Studies on 'Infantile Cirrhosis' and on the effect of cirrhogenic toxin on the progeny.

by

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C O N T E N T S.

P A R T I.

Experimental Studies.

(1) General Survey and plan for Investigation.

General Survey and plan for Investigation.

Infantile Cirrhosis.

Synonyms. Infantile biliary cirrhosis, infantile liver, Brahmin Liver, Subacute toxic cirrhosis of infants.

The disease was first brought to the attention of the medical world by Sen in the year 1882 in a paper read at the Calcutta Medical Society. He described the disease as a peculiar enlargement of the liver which he had observed in infants in Calcutta since 1881 ; but which was not prevalent in Central and Northwest Provinces. In 1888 Gibbons read before the same society a paper on the pathological anatomy of the liver of a child who had died of the malady. Since then the disease has been the subject of much discussion among medical men in Calcutta, Madras, and other parts of India.

The gravity of the problem that confronts us can be well imagined when we take into account the high rate of infant mortality due to this disease. According to the Calcutta Health Officer's report, in that city alone more than 700 infants die of it in one year. The high incidence of this disease chiefly among the upper and middle class Hindu families, and its irrevocably fatal termination in spite of well tried remedies has infused in the lay public a dread of the disease.

Investigations on the various aspects of the affection have been extensive; but scope for farther work is by no means exhausted.

Attempts have been made by Ghose J. N. (1894) Sircar N. R. (1894) E. Mackenzie (1894) Sing (1911) Green Armytage (1926) Vaidyanath Iyer (1926) Krishna Iyer (1927) Mukherji (1927) Pandalai (1934) and others to arrive at a conclusion regarding the etiology of the disease from a clinical study of the case at the same time focussing some attention on the nutritional aspect.

Gibbons (1888) Ramachandra Rao (1929) and Radhakrishna Rao (1935-36) have sought for the cause from a study of the histopathology of the liver.

The role played by parasites, bacteria, and protozoa in the causation of the disease has been investigated to some extent and upheld as the probable etiological factor by a few observers, namely Pearse T. F. (1909-1910) Chandra Lahiri (1936) Castellani and Chalmers as well as by Manson-Bahr.

Mukherji (1927) was the first to investigate the epidemiology of the disease with a view to arriving at the etiology. Recently Narayanamurit and Tirumurti (1938) Ramachandra Rao and Srinivesa Rao (1939) have continued their investigations on similar lines; but their findings have not been published as yet.

So far with the sole exception of the work of Razek approach has not been made to solve the problem of etiology from the experimental research.

The literature pertaining to the study of the subject is voluminous and the statements sometimes contradictory and confusing; but on the most essential points they are unanimous.

The present experimental investigations are based on the few important, relevant, and authentic facts gleaned from the medley of information dealing with the various aspects of the disease. A scrutiny of the accompanying table will help one to appreciate the value of this mode of access to the problem.

Scope of the investigations.

The following considerations present themselves as facts of fundamental importance for studying experimentally the etiology of infantile cirrhosis as observed in India.

A. Familial incidence of the disease.

Successive children born to the same mother succumb to it one after the other. This is a feature which suggests an antinatal cause acting through the mother. Experiemntal study on the possibility of placental permeability to cirrhogenic toxins, and the effect of such toxins on the foetus.

B. The relation between the occurrance of infantile cirrhosis and breast feeding;

taking into consideration the common vogue of breast feeding in India. Experimental study

on the effect of cirrhogenic toxin administered to the mother, on the liver of the suckling.

C. The factors that favour the occurrance of the disease.

Diet chiefly of polished rice to the exclusion of animal proteins and purine bases in any form.

(1) Its peculiar geographical distribution.

The disease is found mainly in the rice growing districts in India.

(2) Ethnological and social consideration.

It is found chiefly in the families of the upper and middle class Hindus, particularly in the Brahmin community. Their staple diet is polished rice.

(3) Dietetic habits of the people.

In south India it affects almost exclusively children whose parents are vegetarians and do not take egg, meat or fish.

Experimental production of liver damage in sucklings whose parent is fed on polished rice and vitaminsand is subjected as well xx to the action of cirrhogenic toxin.

D. The factors that oppose the development of the Disease.

Diet of Cereals other than rise, or a diet of polished rice with animal proteins.

(1) Absence of the disease in districts where there is only dry cultivation i.e. cultivation of wheat and millets.

(2) The immunity from the disease enjoyed by the poor class whose staple diet is the cheaper millets and parboiled unpolished rice.

(3) Dietetic habits. Mohammedans, Europeans Angloindians and Indian christians who take a mixed diet of meat and rice are less susceptible to this disease.

Experimental determination of immunity from liver damage in sucklings whose parent is fed on polished rice vitamins and nucleic acid, and is subjected as well to the action of cirrhogenic toxin.

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Tables showing data relating to "infantile cirrhosis" according to different authors.

Date of Publication.	Author.	Districts where the disease is observed.	Age Incidence.	Social Status.	Dietetic Habits.	Etiology according to the Author.
1891	Gibbons.J.B.	Calcutta and other parts of Lower Bengal.	3rd Month till end of 2nd year.Usually under 1 year.	"Among those of the well to do classes of Hindus and Mohammedans.I have never seen a case in a European or Eurasian child".		There are good grounds for believing that the disease is brought about by irritation produced by products of faulty digestion and that the cause lies in unsuitable food.
1894	J.N.Ghose	Calcutta and other parts of Lower Bengal.	Under 1 year seldom after 3 years.Duration 3-9 months.	Mohammedans and Eurasians suffer less than the Hindus.Hardly any among Europeans.	Mohammedans and Eurasians feed their children with animal broth.The Hindu Mother always nourished her children from her own breast. In those families where the disease prevailed I noticed a few children excape apparently from being nourished by healthy wet nurses.	(1)Predispostion since children get the disease a few days after birth and successive deaths of a number of children in the same family. (2)Suckling the child when pregnant. (3)Irregular and over feeding.
1894	N.R.Sircar		95% before 2nd year, is completed.		I have on record three ladies.In one of whom all the children who were fed on breast milk died of the disease whereas in the other two though the children who were fed on breast milk died of enlarged liver, the younger ones who were put on artificial diet have grown up healthy.	(1)Improper food and milk. (2)Climatic condition.
1894	S.Mohan Dass			Health Officers report was quoted. Hindus. Mohammedans. 1891 486 21 1892 546 30 1893 584 29		
1894	Peers Dimmock	He has seen a few cases in Bombay where it does not seem to be so prevalent as in Calcutta.The disease doubtless occurred all over India but some localities were affected more.e.g.Canara and Calcutta.				

Continued

Date of Publication.	Author.	Districts where the disease is observed.	Age Incidence.	Social Status.	Dietetic Habits.	Etiology according to the Author.
1894	E.Mackenzie V.H.A.S.	Canara.S.W. Coast of Bombay presidency 20 cases per year.	From birth up to 3 or 4 years.	"The disease has been confined to Brahmin children or in those approaching in habit and mode of living to the Brahmins, but why it should be peculiar to them is not clear. A single instance was not found in Sudra, christian or musselman".	Brahmin women in child bed adopt a diet which may conduce to the disease in the new born infant in whom it has been seen. They restrict themselves to the use of a strong decoction of black pepper to allay thirst, abstaining from liquid of any other kind and as food use balls made up of boiled rice, ghee and coarse sugar.	It is possible the pepper acting through the mothers milk may cause irritation of the liver.
1894 and 1895	Ghose J.N. and Mackenzie.	Calcutta and districts of Bengal.no.400.	Under 1 year seldom above 3 years.	Children of wealthy and middle class Muhamadans and Eurasions suifer less than Hindus.	In those families where the disease prevailed I noticed a few children escape apparently being nourished by healthy wet nurses.	The habit of suckling their children when pregnant. Predisposition.
1896	Gibbons.J.B.	Calcutta and probably other parts of India.				I put forward the view that the disease was due to food. Improper food and faulty digestion.
1896	Nil Ratan Sircar.	Calcutta.			I have on my record three ladies of one of whom all the children fed upon breast milk died of enlarged liver; where as in the two others though the elder children fed upon breast milk died of the same disease. The younger ones who were put upon artificial food soon after their birth have grown up with little trouble.	A proper selection of articles of diet would prevent the disease in many cases and would arrest it in others.
1909	Pearse.T.F.	Bengal and Madras.	6 months to 2 years.	In 1907 (Hindus ..502)Mohamedans ..134 Rich as well as poor.	It is difficult to ascribe the disease to errors of diet.	We can most reasonably conclude that it is a parasitic disease.
1910	Idem	Bengal	6 months to 2 years.	In 1908 Deaths due to this disease 727 Hindus ..596 Mohamedans ..124 Others. .. 7		Parasitic in Origin.

Continued

Date of Publication.	Author.	Districts where the disease is observed.	Age Incidence.	Social Status.	Dietetic Habits.	Etiology According to the Author.
1910	Castellani & Chalmers	Calcutta and other parts of India.	Under 1 year.	Hindus and Mohammedan.		Possibly it is identical with the infantile splenic anaemia caused by L. Infantem.
1911	Singh.B.	Bengal and United Provinces.	6 months to 2 yrs.	Hindu families.	Three of the cases were on Mothers milk and fourth on cows milk.	Some connection with dietery.
1919	Castellani & Chalmers B.J.	Calcutta and other parts of India.	Under 1 year. Duration 3-8 mths.	Hindu and Mohammedan children.		Requires reinvestigation with a view to deciding whether it also is a variety of KALA-AZAR.
1922	Byam.W. & Archibald R.G.	Calcutta and other parts of India.	Infants three to eight months duration.			The condition appears to differ from portal cirrhosis in its much more rapid course.
1923	De Costa R.F.W.		13 months	Well to do Hindu family.		
1926	Vaidyanath Iyer.A.S.	Tellicherry (West Coast).	Under 6 mths.1. 6-12 mths. 25. 1-2 yrs. 19. 2-3 " 13. above 3 yrs. 1.	Brahmins 18. Hindus 22. Mohemmedans. 15. others. 4.	Prevalent among both vegetarians and non-vegetarians.	Faulty digestion.The early use of starchy food is a causative factor. The addition of alittle rice food to the diet agravates the symptoms where as with holding it for a few days brings improvement.
1926	Green Armytage	Calcutta.	Between 5 months and 3½ years.	Anglo Indians or European Hindus and Mohommedans.	1) If the mother feeds incorrectly before the birth of the child she may dispose the child to develop this disease. If the Mothers diet is deficient in vitamins the quality of her breast secretion suffers. 2) Auto intoxications in the Mother. 3) Improperly fed and overfed children. 4) Milk of cows fed on dry fodder	
1927	Krishna Iyer.M.A.	Tamil districts and eastern districts of South India.		Brahmins High caste Hindus and Mohammedans.		Early administration of starch and fatty food. The use of patent and tinned foods.

Continued

Dateof Publication.	Author.	Districts where the disease is observed.	Age Incidence.	Social Stutus.	Dietetic Habits.	Etiology according to the Author.
1927	Manson Bahr.	Bengal, Madras, Bombay presidencyand the United Provinces.	Children under 1 yr. As a rule about the 7th or 8th.mth.Duration 3-8mths.Rarely within a few days of birth.	The children of the well to do are more frequently affected than those of the poor.It is found to be more prevalent in Hindu than Mohammedan children.In Calcutta 1891-1893 inclusive - it caused 1,748 deaths. Hindus - 1616 Mohammedans - 80. Eurasians and others - 52.	The disease is especially apt to occur in grossly over fed and pampered children.	Possibily a proportion of cases are infantile Kala-Azar.A complete change of wet nurse and food may have a beneficial effect. When ever possible the latest baby in the family in which several cases of this disease have already occured should immediately be removed from the Mother and artifically nursed.
1927	Mukherji	Bengal,Calcutta. and various districts Madras.Arcot.Salem. Trichinopoly.Madura.Ramnad. Tanjore, Pudukota. Mysore. Rarely from U.P. or Bombay.	6 months to 2 yrs.	Bengal.Hindus - 240 Muslims- 51 Christian- 1 Madras.Tamils - 76 Telugu - 5 Malayale - 1 Rare among christians and angle-Indians.	Bengal.The charactersitic of the diet is excess of carbohydrate. The chief meals consist mainly of rice milled. Very few vegetables or fruits. Madras.Principle food rice.Brahmins and High class Hindus are vegetarians.	
1929	Rolleston & McNee.	It occurs expecially in, but not confined to Calcutta.	Young children.	Attacks chiefly Hindus.		It has been thought to depend on irritating bodies in food, especially as the nursing Mothers restrict themselves to a dry diet and take a decoction of black pepper.
1929	Ramachandra Rao.M.G.	Pudukkotah State.		Brahmin families.		Quick succession of Pregnancies.
1931	Bhattacharji S.P.	Very large number of cases in Bengal also in Madras.			In a number of cases in which the exact dieteries were studied in detail there was excess of sugar and deficiency of protein.	
1931	Nandi.P.					Deficiency of anti-scorbutic factor.
1931	Sankara Iyer.	Mysore State.	9-15 month.	Out of sixty cases 90% were among Brahmins of the lower and middle class.		Health of the Mother undermined by rapidly following pregnancies.

Continued 10.

Date of Publication.	Author.	Districts where the Disease is observed.	Age Incidence.	Social Status.	Dietetic Habits.	Etiology acording to the Author.
1931	Cothoskar	All over India.		Christian women are more healthy & I have never seen a case of Infantile Biliary Cirrhosis among their children.		1)Boiling the milk & removing the cream. 2)Normal protein but deficient sugar count of milk.
1931	T.Bhaskara Menon	Calcutta,Bombay, Madras.	6 months 2 years.	Brahmin children.		1)Antinatal cause acting slowly after birth. 2)Toxin excreted through milk. 3)Microbic origin. 4)An umbilical vein cirrhosis.
1934	Pan dalai N.G.	Calcutta,Bombay, U.P.Malabar,Travancore & Mysore Cities and Towns than villages.	6 months to 3 yrs. begins between 6th & 8th.mth.	The children of rich & middle classes.The disease is common among Hindus,less among Mohammedans & rare among Anglo-Indians.		1)Vitamin deficiency operating through mother. 2)Over worked and exhausted liver. 3)Early administration of starchy food. 4)Toxins from mothers milk or from intestines. 5)Congenitally insufficient liver. None of these are satisfactory.
1934	Tirumurti T.S.& Rad Hakrishna Rao M.V.	Quoting Sarma Kumbakonam			Vegetarians families (They take neither eggs, or fish nor meat).	He considered that the prevalence of the disease in vegetarian families is due to early administration of starchy food, and probably due to an increased amount of sugar inthe breast milk of vegetarian mothers.

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Date of Publication.	Author.	Districts where the disease is observed.	Age Incidence.	Social Status.	Dietetic Habits.	Etiology according to the Author.
1934	T.S.Tirumurti & M.V.Radhakrishnan.	Eastern plains of Madras presidency lower Bengal.U.P. and West Coast.	6 months to 3 years.		Our experience in the circars shows that the disease is commonly seen in the Hindus esp. the Brahmins and vaishnavites of the middle and rich classes. During the last 2 yrs.no case of this disease was seen by us in European,Anglo-Indian chris: or Mohamms:families.In the Madras Presidency the dis: is more prevalent in the S.dis+ amongst Brahmin families.	1)Dietetic erros. 2) Virus. 3) Subacute toxic infection.
1938	M.V.Radhakrishnan Rao.	India chiefly.Reported from Mexico and from China.	6 months to 3 years.			The disease is due to the action of a toxin on the liver (a toxin contained in the diet & indigenous to India)which circulates in the maternal blood in intra uterine life, &discontinued to be given to the child after birth.
1936	S.Chandra Lahiri.					Subacute infection especially by B.Coli or streptococci in an already devitalized&over taxed liver.
1938	Phillipe Rezek (Vienne)	It is not found in the whole of India but is concentrated in Bengal in the surroundings of Madras. South India and round about Vizagapatam.	Commences between 6th & 8th.mths.of infancy rarely after the 12th mth.and the children die almost always after an illness of 4 to 8 months.	The disease attacks mostly children of the middle class Hindus.Rich Brahmins & rarely the families of poor Hindus more rarely still among the Mohammedan and never the Anglo-Indians or Europeans.	Quoting Radhakrishna Rao-: 'The Brahmin in the neighbourhood of CALCUTTA,VIZAGAPATAM and round about MADRAS are in the habit of feeding their children with rice as early as possible! Quoting Forsyth)-: 'That it is due to early administration of spices'.	The ingestion of spices during pregnancy and lactation can injure the liver of the foetuses as well as that of the Infant.

P A R T I.

EXPERIMENTAL STUDIES.

(2) Experimental study on placental permeability to cirrhogenic toxins.

Liver damage in the feotus due to an antinatal cause acting through the mother.

Review of literature on the familial incidence of infantile cirrhosis, and on the antinatal factors in its etiology.

Cirrhosis of the liver in infants whether it be the infantile cirrhosis so common in India or the rarer cases seen in Europe and Amercia deserves special consideration in virtue of its tendency to affect children of the same mother. This peculiarity has been noted by most observers. It was Sen (1882) who first suggested "Some constitutional predisposition or dyscrasia inherited by the children which under any of the least exciting causes gives rise to this disease. J. N. Ghose (1894) noted that the children of some parents were particularly liable to the disease and has recorded a case where 14 children of the same mother had succumbed to it. Mackenzie (1894) attaches great importance to the fact that "Brahmin women during pregnancy adopt a diet which may conduce to the disease in the new born infant." Nil Ratan Sircar (1896) quotes the occurrence of the disease in successive children of three women. Green Armytage (1926) considered deficiency of vitamins in the diet of the mother depressing the endocrine system of the foetus as one of the causes. Bhaskara Menon states that the striking feature of the disease is a tendency to affect children of the same mother and suggests an antinatal cause. Sankara Iyer (1931) from a survey

of 60 cases of infantile cirrhosis considers that the health of the mother which is undermined by rapidly following pregnancies as the cause of the disease. Tirumurti and Radhakrishna Rao (1934) report several cases in which children of the same mother had died of the disease. They advocate a thorough examination of the mother of the child. Pandalai (1934) suggests a congenitally insufficient liver with inadequate defence powers. Phillepe Razek is of opinion that the ingestion of species during pregnancy and lactation can injure the liver of the foetuses as well as that of the infant.

Experimental study on plancental permeability to cirrhogenic toxins.

Introduction. How far various substances in the maternal organism may affect the foetus in utero has been a matter for discussion for years,and it continues to be so still. The consensus of opinion appears to favour the physical process of diffusion according to which the plasma on either side of the permeable membrane should contain the same amount of all soluble substances. The transference of the soluble substances takes place through the vascular chorionic villi of the foetus which are bathed in the maternal blood in the chorio-decidual or intervillous spaces. Cunningham R. S. (1922) concludes from his experiment that the chorionic villi exert a specific regulating mechanism. But this specific secretory

theory is denied by most observers. Yoshitaka Shimidza (1922) from his experiments on the permeability of the placenta of albino rat and white mouse to various dyes inferred that lipoid soluble dyes regardless of their acid or basic characters generally pass the placenta and that the placenta acted as an ultra filter to the various dyes. A similar view is held by Wislocki (1931) E. D. Plass (1912) Britton (1930) and Helen Bourquin.

Of the many chemicals which permeate through the placenta and act injuriously on the foetuses lead occupies a place of pre-eminence by reason of its extensive use in industries. Weil Halle, A. Abaza and Meunier (1939) report a case of renal dwarfism due to lead intoxication during pregnancy. Speert (1940) in his experiments on the transmission of sulphanilamide through the placenta, and its effects on the foetus and new born, found the same sulphanilamide content in the serum of the mother and the foetus.

The following experiments were conducted in order to find out

1. If a cirrhogenic parenchymal toxin such as carbon tetrachloride or a cirrhogenic mesenchymal toxin such as the alkaloid senecionine can diffuse through the placental barrier.
2. Whether the epithelium of the chorionic villi has a specific regulating mechanism in the transmission of dissolved substances to the foetus.

3. In case such a mechanism does exist whether the cirrhogenic toxins in their passage through the placenta could destroy the mechanism in such a way as to allow the passage of colloidal dyes which normally do not pass through the placenta.

4. The effect of toxic doses of carbon-tetra chloride and senicionine on the pregnant rat and on the foetuses.

Animals used for the experiments.

For all these experiments the albino rat was the animal of choice. Many consideration had to be entered into in the choice of this animal; the foremost being that these animals are prolific breeders, breeding at the rate of four litters in one year. They are easy to procure and can be handled dexterously with a little experience. Rats are of immense value especially in experiments dealing with dietetic factors, as they are omnivorous. It has been expressed by Ophuls (1910) Findlay(1924) and others that it is hazardous to evaluate experimental cirrhosis in rabbits since the majority of them suffer from coccidosis with varying degrees of chronic hepatitis. As for guinea pigs their period of gestation is rather long (70 days), and the animals do not withstand carbontetra chloride toxaemia well.

Rectangular cages of galvanized iron with wire netting in front, and with a sliding lid were

used to house the rats. Two females and one male rat were allowed for each cage, and vaginal examination was carried out with a flexible wire loop every morning. The presence of the seminal plug within the vagina was taken as evidence of copulation, and the period of gestation was calculated from the date when the vaginal plug was seen. The normal period of gestation is 22 days but as it takes 12 to 24 hours for the plug to disappear from the vagina, a rat may litter one day earlier than the calculated period. In most cases on the 12th or 13th day of gestation a trace of blood appears in the vagina (Placental sign) The females were isolated in separate cases as soon as copulation had taken place, and enough hay was left in the cages for the animals to make their nests.

The animals were fed daily on oats and dog biscuits soaked in diluted milk, together with cod liver oil, marmite and cabbage twice a week.

Cirrhogenic toxins used. In all the following experiments carbon tetra chloride and the alkaloid senecionine were used as the toxic agents to produce liver damage in the experimental animals.

Carbon-tetra-chloride has been studied as a cirrhogenic toxin by numerous observers, and a review of the literature on liver damage due to this agent is given in the thesis on "carbon-tetra-chloride in relation to liver regeneration" by Cameron and Karunaratne (1936). It is especially suited for

work on rats as it very rarely produces any necrosis at the site of injection if it is given subcutaneously in between the shoulder blades. In the experimental animals especially in dogs and rabbits it was found to produce central necrosis of the liver parenchyma followed by marked central fibrosis (Gardner, George H., et al. 1925), a type of cirrhosis classified as toxic cirrhosis by Mallory (1911). Carbon tetra chloride has also been observed to produce toxic cirrhosis in man (Poindexter and Greene, 1934) a condition some what analogous to "infantile cirrhosis" of India.

Senecionine. It has been known for some time that cirrhosis of the liver was produced in cattle after they had been fed on fodder contaminated with common ragwort or senecio jacoboea. Such cases have been reported from New Zealand, Nova Scotia, South Africa, as well as from England. Eleven cases of cirrhosis have been reported by Willmot and Robertson (1920), the majority of which occurred in children as a result of accidental ingestion of the seeds from this plant along with wheat. The liver damage in these cases was said to be due to the presence of an alkaloid in the plant. Different alkaloids have been extracted from various plants, belonging to this genus. The toxicity of the alkaloid has been demonstrated by feeding experiments on cattle and on laboratory animals. As regards the action of the toxin it was expressed by Watt and Brandwijk (1932) that the

parenchymal cells of the liver were first affected, and that the vascular lesion was secondary to the parenchymal degeneration. Later experiments by Davidson disproves this theory as he observed that the primary change occurred in the branches of the hepatic vein which resulted in their rupture.

Material and Methods of Study.

Eleven pregnant rats were used for these experiments, three of which were kept as controls. Pure undiluted carbon-tetra-chloride, and senecionine in a one per cent solution in distilled water acidified with acetic acid were used as the cirrhogenic toxins. The alkaloid senecionine was kindly supplied by Dr J. J. Blackie. A solution containing one gramme of trypan blue and one gramme of orange G. in 100 c.c. of distilled water was used to test the damage to the placenta; but tests on control animals proved the valuelessness of orange G. for this purpose, and its use was dispensed with in the later experiments. For injections of carbon-tetrachloride and senecionine, tuberculin syringe fitted with a No. 16 needle was used. The tissues were fixed in 10% formol saline. Unstained frozen sections were studied to find the distribution of the dye in the tissues. Sections of tissues embedded in paraffin and stained with Meyer's acid alum haematoxylin were used to study the damage to the liver of the foetus and that of the mother.

the foetuses' were examined macroscopically and microscopically. It was observed that the foetuses were all viable, and there was no evidence of death in utero.

Results.

In control animals.

Effect on the mothers. Similar changes were seen in the tissues of the two animals. The whole body including the sclera was stained light blue. As for the organs the liver had taken the deepest hue of blue and the spleen was of a lighter blue colour. The cortex of the kidney was tinted deep with the dye; but the medulla was of normal colour. The mucous membrane of the stomach and intestine was intensely blue; so also was the intestinal content. The bulk of the stomach content was free from stain, but an outer layer to a depth of about 2 m.m. was stained light blue. The excretion of the dye was obviously taking place through the mucous membrane of the alimentary tract. The urine in the bladder was deep orange in colour; but this tint could not be made out either in the skin or in the organs in the presence of the dominant colour of trypan blue.

Microscopically unstained frozen sections from the different organs showed the concentration of the dye in certain cells only. In the liver the histiocytes in the portal tract and the Kupffer cells of the sinusoids were stained blue. In a section of the kidney the

colour was seen to be confined to the cortex in the epithelial cells of the convoluted tubules; but it was not being excreted through the urine. Littoral cells and the pulp syncytium of the spleen were also tinted with blue.

The foetuses were decidedly not stained with trypan blue. Orange G.is reputed to pass easily through the placenta; but the stain, if there was any, was too faint to be made out by naked eye examination.

Placenta. The maternal portion of the placenta composed of the decidual cells and the foetal portion limited to the chorionic epithelium as well as the foetal membranes were stained blue with the dye.

In the experimental animals (see coloured plate)

In the mothers the distribution of the dye in the various organs was similar to that obtained in the control animals. In addition, changes in the livers due to the injection of the two toxins were also noted. The liver of the animals which had carbon-tetra-chloride appeared slightly swollen, with an exaggeration of the lobular pattern. Surface was pale yellow in colour, mottled with red. The organ was soft and easily friable.

Microscopically necrosis of the liver cells affecting a third to half the lobule about its centre was observed. The cells in this region took a homogenous salmon pink stain with Mayer's haemalum eosin, and sections stained with Sudan III. showed numerous droplets of fat. The nuclei of these cells had either dis-

Fig. I. The animal had received 0.3 c.c. of Carbon-tetra-chloride on the 18th day and 10 c.c. of 1% Trypan blue on the 19th day of gestation respectively. It was sacrificed on the 20th day of gestation. All the tissues of the animal were stained blue. The maternal portion of the placenta as well as the foetal membranes were stained blue. But the foetus and foetal half of the placenta were not stained. (See coloured plate).

appeared or were undergoing karyorrhexis and lysis. The Kupffer cells and endothelial cells were comparatively little affected, and there was an apparent increase in the number of these cells. Frequently around the area of necrosis was seen a single layer of liver cells ballooned out to an enormous size with central pyknotic nuclei but no stainable cytoplasm, a condition often referred to as hydropic degeneration. The cells surrounding the portal tract were well stained, and the structures in the sheath were normal.

With senecionine toxaemia the naked eye and microscopic changes observed in the liver were slightly different. The organ appeared swollen, congested and haemorrhagic. Microscopically central necrosis of the parenchyma, and haemorrhagic exudation in the necrosed area were the obvious features. The central veins had ruptured in many of the lobules, and they together with the distended sinusoids contributed to make up the picture described by Davidson and others as "lagoons of blood". The necrosed cells in the centre of the lobule did not stain well either with the haematoxylin or with eosin. The disintegrating nuclei and the homogenous cytoplasm could be faintly identified. There was moderate proliferation of endothelial cells, and infiltration with polymorphs and lymphocytes in the necrotic area. The cells in the peripheral zone around the portal tract and the structures in the tract were unaffected.

The foetuses as in the control animals were not stained with the dye; but microscopically the livers

of the foetuses whose mother had carbon-tetra-chloride showed the following changes. The parenchymal cells were much swollen, and the cytoplasm vacuolated to such an extent that very little of it except the cell outline, and a narrow margin around the nucleus was actually stained. The nuclei had disappeared from many of the cells, and of those which were still left a large number appeared distorted and poorly stained. The distribution of the haemopoetic cells was normal, and the cells themselves were healthy and well stained. The sinusoids were moderately open and contained red cells. The hepatic and portal vessels were distended.

The microscopical appearance of the liver of the foetus whose parent had senecinonine was slightly different. There was a moderate depletion of the mesenchymal or the haemopoetic cells. As compared with the normal foetal liver these cells were scarce. The cytoplasm of the parenchymal cells was vacuolated and the cells were swollen. Many of the nuclei had disappeared, leaving only the bare outline of the cells. But those which were present took the normal nuclar skin. The blood vessels and sinusoids were markedly distended with blood.

Permeability of the placenta to toxins from the foetus to the mother.

Control. One albino rat, 18th day of gestation. The abdomen was opened under ether anaesthesia. The left cornu of the uterus was exposed and drawn out of

the opening. The head, neck and limbs of the foetuses could be identified through the translucent uterine wall. One of them was gripped gently between the forefinger and thumb of the left hand and by means of a tuberculin syringe fitted with No. 18 needle 0.25 of a 1% solution of trypan blue was injected into it subcutaneously between the shoulder blades, the needle being passed at an obtuse angle through the uterine wall. There was no escape of amniotic fluid through the needle track. A second foetus was injected in a similar manner in-utero with the dye. The uterus was replaced into the abdomen, and the wound was closed with interrupted sutures. The animal was allowed to litter and then sacrificed.

Experiments: Four experiments were performed ; two with carbon-tetra-chloride and trypan blue, and two with senecionine and trypan blue.

1. Albino rat, 19 days pregnant. Abdomen was opened under ether anaesthesia, and the right cornu of the uterus was drawn out of the opening. Three foetuses were injected each with 0.1 c.c. of trypan blue and 0.1 c.c. of carbon-tetra-chloride in a single dose. Three more foetuses from the left cornu were similarly injected. The uterus was replaced, and the abdominal wound closed with sutures.

2. Albino rat 19 days pregnant. Five of the foetuses were injected each with 0.075 c.c. of carbon-tetra-chloride and 0.075 c.c. of trypan blue solution. Abdominal wound was closed.

3. Albino rat 19 days pregnant. Five of the foetuses were injected each with 0.15 c.c. of senecionine and 0.05 c.c. of trypan blue. The animal died on the second day.

4. Albino rat 19 days pregnant. Four of the foetuses were injected with 0.125 c.c. of senecionine and 0.125 c.c. of trypan blue. The parent rat was killed on the second day.

Result.

The control animal littered on the second day. Litter of nine, of which two were stained intensely blue, and the rest were of normal colour. All the young as well as the mother were healthy. There was no suggestion of the dye having passed from the foetus to the mother. The mother, the two young ones which were injected and two uninjected ones were sacrificed on the same days. Naked eye and microscopic examination of the tissues of the various organs of the mother failed to reveal the presence of trypan blue. There was no stain in the tissues of the uninjected foetuses, whereas in the two foetuses which were injected with dye all the organs as well as the skin were stained blue.

Result in Experiments.

Experiment I. The rat died the day after the operation. The uterus was laid open and it was found to contain eleven dead foetuses of which six were stained deep blue. The placenta of the injected foetuses

showed the blue stain in the foetal portion only. The maternal portion was not stained at all. The tissues of the mother were free from stain, but the liver was pale yellow in colour, mottled with red suggesting moderate fatty degeneration. Microscopical examination confirmed this change. The central and half of the midzonal region in sections stained with Sudan III. showed marked fatty degeneration of the parenchymal cells. Early necrosis of a few cells around the central vein were also observed. The spleen was swollen and congested but there was no stain in the littoral cells or in the pulp syncytium.

Experiment II. Animal littered two days after the operation. Litter of two, both of which died two hours later. The parent was sacrificed. The uterus was found to contain eight foetuses. Five of the foetuses which had been injected were dead. Of the uninjected three, two were alive and one was macerated. Liver changes in the mother were similar to that found in the previous case (See coloured plate).

Experiment III. The animal died on the third day after the operation. There were ten fully developed dead foetuses in the uterus of which five were stained blue. None of the feotuses showed signs of having died before the mother. The placenta of the injected foetuses were stained only in the foetal half. The parent animal was not stained. The liver was slightly below average size with sharp edge. It was pale yellow in colour and speckled with purple spots. Microscopically

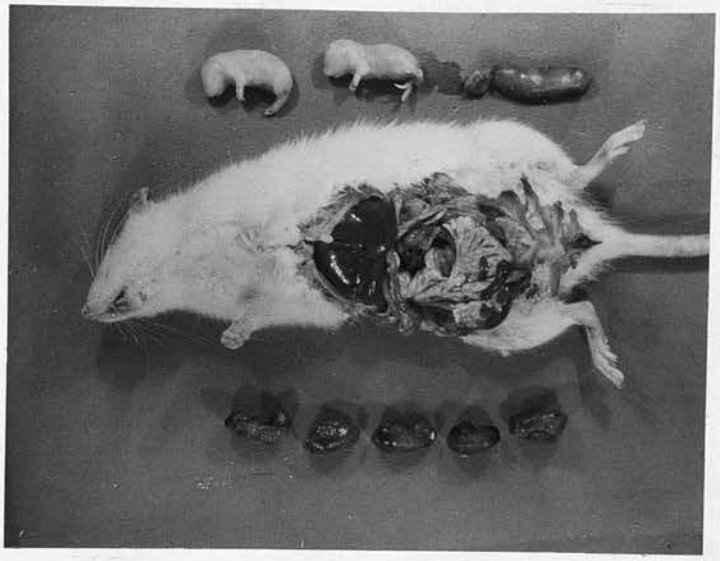


Fig. II. Exp. II. Five of the foetuses were injected each with 0.075 c.c. of Carbon-tetra-chloride and 0.075 c.c. of 1% Trypan blue on the 19th day of gestation. The animal delivered two live litter both of which died within two hours. It was sacrificed on the same day. The five injected foetuses were still stained deep blue, but neither the mother nor the other live foetuses were stained. Micorscopical examination of the liver of the mother showed extensive fatty change due to the permeation of the carbon-tetra-chloride from the foetus to the mother.

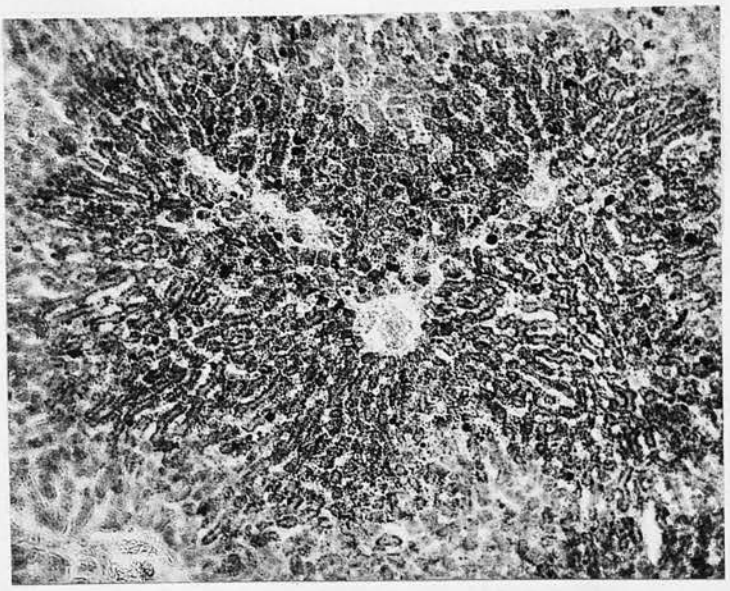


Fig. III. Liver of the pregnant rat in experiment II. showing extensive fatty change in the parenchyma. Frozen section Sudan III. x 85.

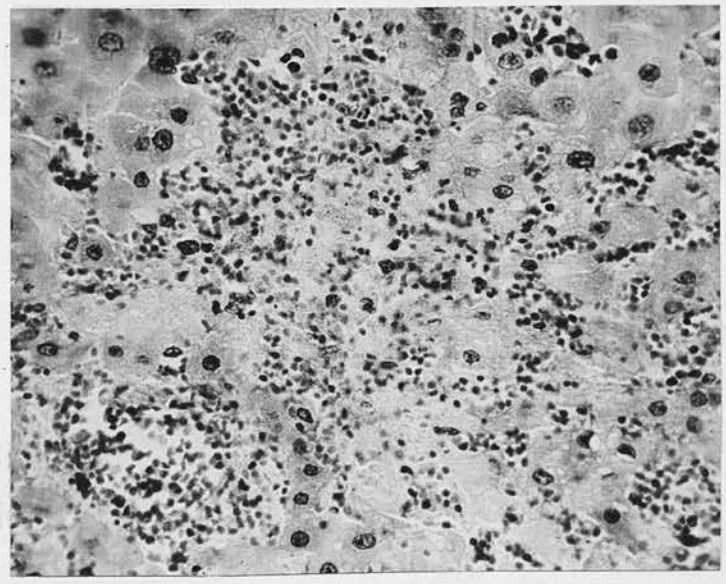


Fig. IV. Liver of pregnant rat in experiment III. On the 19th day of gestation five of the foetuses were injected each with 0.15 c.c. of 1% solution of senecionine and 0.05 c.c. of trypan blue. The animal died two days later. The liver shows necrosis of parenchymal cells and intense congestion of the sinusoids. Pools of blood are observed in the necrotic area.

H and E x 300.

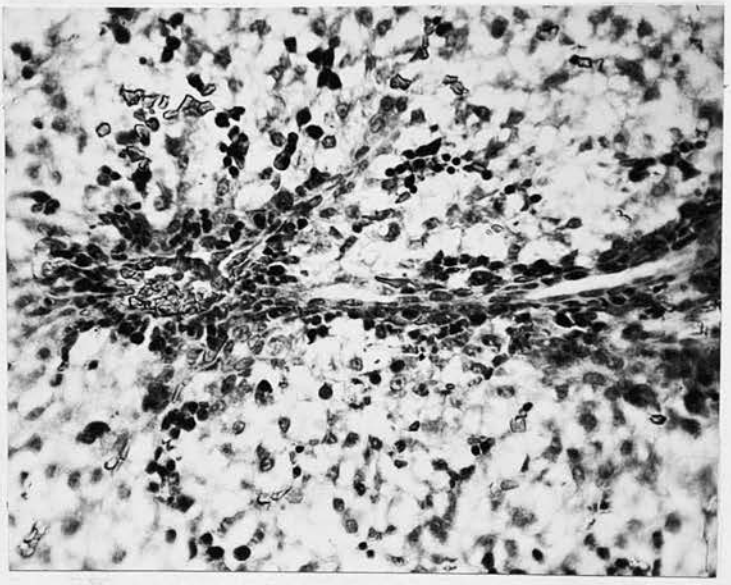


Fig. V. Liver of the foetus whose mother had received 0.3 c.c. of carbon-tetra-chloride on the 18th day of gestation. The parenchymal cells are swollen and hydropic except for a narrow margin of cells around the portal tract. The haemopoetic cells are not affected.

H and E x 300.

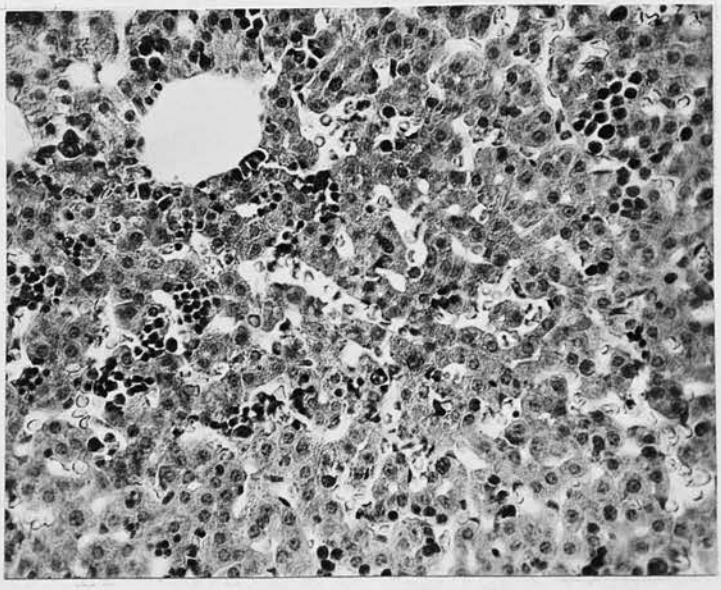


Fig. VI. Liver of the control foetus whose parent received injection of the dyes only. Anastamosing cords of hepatic cells are seen with well stained cytoplasm and large nuclei. Islands of haemopoesis are distributed throughout the lobule.

H and E x 300.

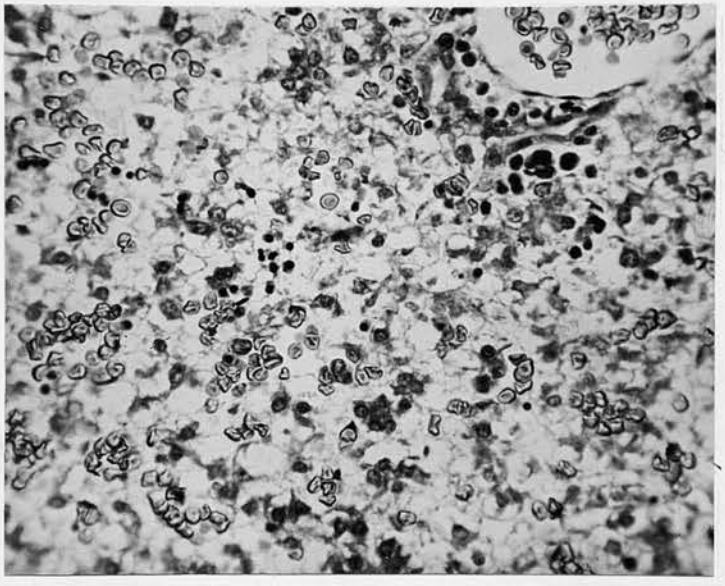


Fig. VII. Liver of the foetus whose mother received 15 mil. grams of senecionine on the 15th, 17th & 19th days of gestation. The number of haemopoetic cells are less than in the control. The parenchymal cells are swollen vacuolated and the cytoplasm has not taken the stain. The sinusoids are distended with red cells. H and E x 300.

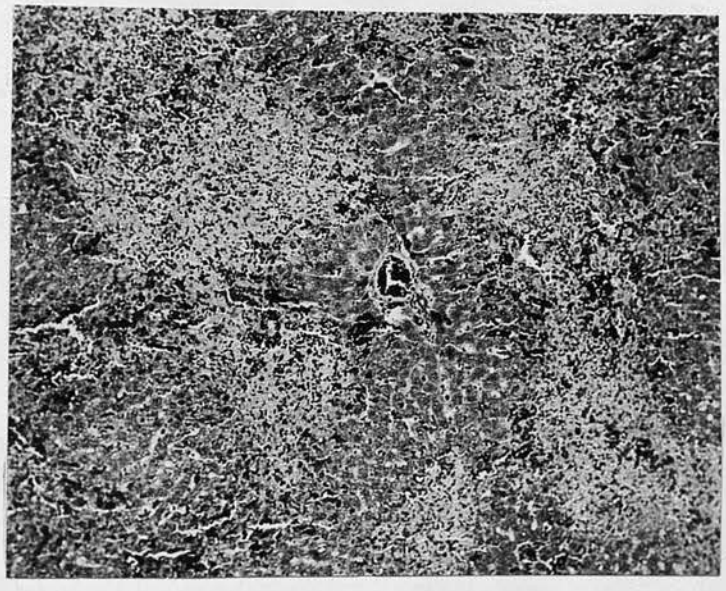


Fig VIII. Liver of rat No. 58. which had received 10 mil. grams of senecionine. Extensive central necrosis of the lobule is seen. The necrotic areas are infiltrated with polymorphs and lymphocytes. There is marked congestion of the sinusoids.

H and E x 85.

the parenchymal cells showed extensive degenerative changes and necrosis chiefly around the central vein. The liver cells appeared homogenous with opaque basophilic nuclei. Karyorrhexis and lysis of the nuclei were frequently seen. In the centre of the lobule many of the liver cells had disappeared leaving wide spaces filled with blood. There was slight inflammatory cell infiltration at the portal tracts. The wall of the blood vessels were oedematous and friable. Spleen was swollen, tense and purple. Stomach showed haemorrhagic infarcts.

Experiment IV. The animal was killed on the second day. The uterus contained ten foetuses. Of the four injected foetuses three were alive and one was dead. The other six uninjected foetuses were also alive. The organs of the mother were free from stain, yet degenerative changes were present in the liver, similar to that found in experiment III. but to a lesser degree.

Effect of toxic doses of cirrhogenic toxin on the pregnant rat and on the foetuses.

Material and methods of study.

Twenty two albino rats together with eight controls were used for the following experiment. The period of gestation was calculated from the date the vaginal plug was observed. All the rats including the controls were kept under identical conditions.

Carbon-tetra-chloride toxaemia. Fifteen of the experimental rats were given injections of carbon-tetra-chloride and the injections were started on different days ranging from the date of copulation to the eleventh day of gestation. The first dose in all cases was 0.15 c.c. and the subsequent doses were 0.2 c.c. The injections were given twice weekly, subcutaneously on the back between the shoulder blades well behind the nape of the neck. It was found by trial that injections on the abdominal wall, or at the root of the tail, were often followed by necrosis if the dose was large, whereas if given between the shoulder blades necrosis never occurred even with very large doses. In rats, which suckled their young the advantage in the choice of this site for injection is obvious.

Senecionine toxeamia. Seven of the experimental rats were given injections of 5 milligrammes of senecionine in 0.5 c.c. of acidified distilled water twice weekly. The injections were started on different days from the date of implantation of the placenta i.e. from the 12th day of gestation onwards.

Result.

Controls. Of the eight control animals one failed to implant. The other seven became pregnant and littered on the 22nd day of gestation.

Result of experiment with carbon-tetra-chloride toxaemia. In seven of these animals pregnancy did not follow copulation and there was no placental sign either on the 12th or 13th day. Manual palpation of the uterus through the abdominal wall showed the normal non-pregnant uterus. In cases of successful pregnancy beading of the two horns of the uterus can be made out by palpation. Four animals aborted on the 15th, 15th, 16th, and 20th day of gestation. The animals were off their feed for two days and a bloody foul smelling discharge was noticed from the vagina. In the first three cases no foetus was expelled. On palpation the foetuses were found to have shrunk in size; but could still be felt. In about ten days time they were absorbed and could not be palpated through the abdominal wall. The fourth animal expelled two macerated foetuses on the 20th day of gestation and a few more shrunken and softened foetuses could be felt in the uterus. Four animals littered on the 21st and 22nd day of gestation. In one of these animals all the young were still-births, but without any other evidence of their having been dead in the uterus for long. In two animals many of the litter were born dead, and the rest died soon after. The litter of one animal were all born alive, but died within five or six days.

Result of Senecionine toxaemia. All the animals littered, but three of them were premature. In all the litters there were many dead, or died shortly after

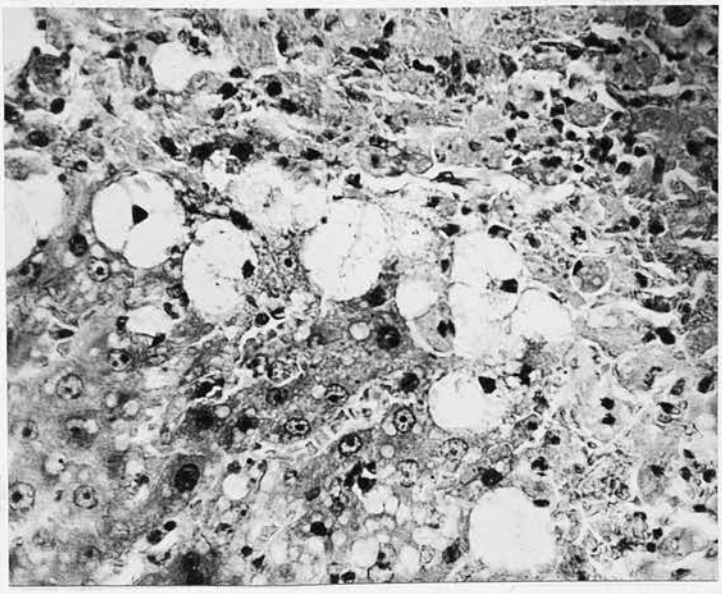


Fig. IX. Liver of pregnant rat which was injected with 0.15 c.c. and 0.2 c.c. of carbon-tetrachloride at intervals of three days. Extensive central necrosis of liver cells fringed by a single layer of hydropic cells is seen. The peripheral zone of cells show slight fatty change.

Fig X. The technique of injecting the cirrhogenic toxin and dye into the foetus while the mother rat is under ether anaesthesia.

birth. In one litter only, some survived for ten days.

Discussion.

Placental permeability. An analysis of the experiments on placental permeability shows that cirrhogenic toxins, carbon-tetra-chloride and senecionine diffuse through the placenta and injure the liver of the foetus extensively. The anatomical consideration that much of the placental blood carrying the normal and abnormal products which have diffused from the mothers blood passes through the hepatic sinusoids before reaching the other organs, assumes special significance in the light of the present study. Trypan blue which forms a colloidal solution with water, although absorbed by the epithelium of the chorionic villi, and the amniotic membrane does not enter the foetus through the circulation as the alkaloid senecionine in true solution does. Trypan blue which occurs in ultra microscopic particles in the solution is taken up by the reticulo endothelial cells throughout the body as well as by the epithelial cells of the convoluted tubules of the kidney and the epithelium of the chorionic villi. Even trypan blue is said to enter the foetal circulation in traces in the rabbit and guinea pig. (Wislocki). As regards carbon-tetra-chloride, its entry into foetal circulation is explained by the fact that all lipoid soluble substances generally pass the placenta. The toxins

Action of Toxic doses of Carbon-Tetra-chloride on pregnant rats, and on the foetuses.

Rat No.	Day of Gest. when injec. was started.	Dose in c.c. during Gest.	Failure to implant by 12th day	Aborted on	No. of still birth	No. born alive	Age at Death
8	7th	0.3	Failed				
10	5th	0.4	Do.				
12	11th	0.45	Do.				
24	3rd	0.75	Do.				
27	1st	0.6	Do.				
32	6th	0.6	Do.				
48	6th	0.35	Do.				
20	8th	0.53	---	15th			
23	3rd	0.75	---	16th			
22	8th	0.55	---	15th			
16	3rd	0.75	---	20th			
7	4th	0.75	---	---	5		
9	7th	0.75	---	---	4	4	Killed
14	10th	0.75	---	---	6	2	2 - 1st day
41	9th	0.6	---	---	---	12	6 - 4th day 6 - 5th day

Action of Toxic Doses of Senecionine on Pregnant Rats and on the foetuses.

Rat No.	Day of Gestation when inject was started	Dose in milligram	Premature birth on	No. of still births	No. born alive.	Age at Death.
49	16	10		4	3	
50	16	10		5	4	
52	17	10		2	1	
53	15	15		1	9	All dead in 10 days.
54	12	15	20	6	0	
57	17	10	19	8	0	
68	16	10	18	-	9	Few hrs.

apparently do not injure the placenta in such a way as to make it more permeable to substances which do not normally pass through, since trypan blue which is actually on the boarder line with regard to its diffusibility does not pass through the placenta after large doses of these toxins have been administered to the pregnant rat.

Permeability of the placenta to substances from the foetal to the maternal circulation is governed by the same laws of osmosis and diffusion. Trypan blue is held up at the chorionic villi and does not pass into the maternal circulation and the foetal membranes are not stained, although carbon-tetra-chloride and senecionine diffuse from the foetus through the placenta and cause changes in the liver of the mother. A dose of senecionine - 1.25 milligrams which is about five times the lethal dose for an adult bulk for bulk when injected into the foetus in utero did not cause its death even after 48 hours. The same dose in a new born litter killed the animal in 24 hours. Apparently the senecionine was being rapidly eliminated through the placenta into the circulation of the mother and the damage to the liver of the parent animal after the injection of either of these toxins into the foetus confirms this finding.

A study of the table on the action of carbon-tetra-chloride on pregnant rats show that in 47% of the rats pregnancy did not follow copulation as

against the 88% of successful pregnancies in the control animals. 27% of the experimental animals aborted between the 15th and 20th day of gestation. Macerated foetuses were expelled in one and in the other three the dead foetuses were absorbed; not an infrequent occurrence in cases of miscarriage in rats. In 20%, half or more than half of the litter were still births, and only one rat out of the fifteen brought forth a litter of live ones, but whose life span did not exceed six days. These abnormalities were not seen in the control rats. These observations indicate the toxic effect of the drug administered to the pregnant mother on the foetuses. It has been demonstrated that carbon-tetra-chloride when given to the pregnant mother in the later period of gestation readily passes through the placenta into the foetal circulation. These experiments show that the placenta is permeable likewise during the earlier period of gestation when the chorionic villi are comparatively thick.

In those cases where the animals were injected with toxic doses of senecionine after the appearance of the placental sign, abortion was a less frequent event; but in every litter there were a number of still births. It follows that either senecionine is less toxic to the foetuses than carbon-tetra-chloride, or that the toxin is not so deleterious in its effects on the full term foetuses. It was observed in the experiments on placental permeability

that larger doses of the same two toxins failed to kill the foetuses when the injections were given later in the gestation period. The other important fact to be noted in these observations is that injection of cirrhogenic toxins into the pregnant rat besides increasing the intra-uterine mortality of the foetuses has a similar effect on the post-natal mortality rate.

In attempting to apply the observed effects on rats to probable effects on pregnant women many considerations have to be taken into account. The longer period of gestation, the nature of the cirrhogenic toxin, and dietetic variations are some of the factors that one has to consider in dealing with this question of the possibility of cirrhogenic toxins in the maternal circulation damaging the liver of the human foetus in utero. Apart from congenital syphilitic cirrhosis, and that due to congenital obliteration of the bile duct, congenital cirrhosis of obscure etiology have been reported frequently in medical literature. Vanvert and Ramond (quoted by Rolleston and McNae) have recorded congenital cirrhosis and ascites in a foetus with enlarged cirrhotic liver which was proved not to be of syphilitic, alcoholi or tubercular in origin. It is not claimed that all cases of "infantile cirrhosis" are of antinatal origin, but experimental as well as clinical evidences are in favour of shifting the responsibility to causes acting through the mother.

SUMMARY.

(1) It was demonstrated experimentally that it is possible for cirrhogenic toxins to diffuse through the placenta of a pregnant rat and cause degenerative changes in the liver of the foetuses.

(2) The passage of such toxins through the placenta does not alter the permeability of the organ to a colloidal dye which normally does not pass through it.

(3) Cirrhogenic toxins injected into the foetuses in utero diffuse through the placenta and produce changes in the liver of the mother ; but the passage of these toxins through the placenta does not affect the organ in such a way as to make it permeable to a colloidal dye.

(4) Repeated administration of cirrhogenic toxins to the pregnant rat before the 12th day of gestation frequently results in failure to implant, or causes increased intrauterine and post-natal mortality. The injection of such toxins after the 12th day of gestation induces premature delivery and increases the post-natal mortality.

P A R T I.

EXPERIMENTAL STUDIES.

(3) Experimental study on the relation between breast feeding and liver damage in the suckling rats with special reference to factors in the diet of the mother that augment or prevent such injury.

Experimental Study on the relation between breast feeding and liver damage in the offspring.

Experimental production of liver damage in the foetus and suckling rats whose parent is fed on normal diet and is subjected at the same time to the action of a cirrhogenic toxin.

Introduction. As early as 1894, when very little was known about the disease infantile biliary cirrhosis Ghose observed from a clinical study of his cases, that there was a tendency for the disease to occur chiefly in children belonging to the Hindu families where the mother always nourished her children from her own breast. He noticed that in families where the children were prone to die of the disease, those that were fed by healthy wet nurses escaped the disease. A similar observation was made by R. W. Sircar (1894). He records the case of three women patients in one of whom all the children fed on breast milk died of the disease whereas in the other two though the elder children who were fed on breast milk died of enlarged liver, the younger ones who were put on artificial diet grew up healthy. Mackenzie (1894) who studied the disease in detail suggested the possibility that gastrointestinal irritants like pepper acting through the mother's milk may be the cause of this disease. Green Armytage (1926) was of opinion that when there is deficiency in the vitamin content of the mother's diet her breast secretion suffers and thus predispose to the disease in the

infant. Manson-Bahr (1927) advised as prophylactic that "wherever possible the latest baby in the family in which several cases of this disease have already occurred should immediately be removed from the mother and artificially nursed." Rolleston and MacNee considered that the etiological factor may be the irritant bodies in the food of the nourishing mother. Pandalai (1934) suggested that toxins from the mothers milk may be one of the causes for infantile corrhosis. Bhaskara Menon (1931) held the view that the disease may be due to toxins excreted through the milk. Radhakrishna Rao (1935) suggested as one of the possibilities that the disease may be due to a toxin contained in the diet and indigenous to India which circulates in the maternal blood in intra uterine life, and is continued to be given through the milk to the child after birth. Phillip Rezek (1938) as a result of his experimental studies arrived at a similar conclusion. He said that the ingestion of spices during pregnancy and lactation can injure the liver of the foetus as well as that of the infant. The importance of breast feeding as a factor in the etiology of'infantile cirrhosis' can be judged from an analysis of the observation of Radhakrishna Rao on the normal dietary of 1100 infants and children in Vizgapatam. He found that of the 1100 infants and children, only 11 were fed on cows milk from birth and 14 on patent foods from birth. Of the remaining 1075, it was found that 184 were fed on breast milk

up to the 6th month, 438 up to the 1st year, 313 up to the second year, 110 up to the 3rd year, 25 up to the 4th year and 5 up to the 5th year.

The dependence of the health of the offspring on the quality of the mothers milk has been recognised from time immemorial, but until recently the effect on the suckling of factors affecting the maternal organism has drawn very little attention from investigators. Very little is known of the mechanism of milk secretion; but from the fact that numerous oils, alkaloids, metals and nonmetals are excreted by the milk it can be assumed, that other factors besides the normal diet of the mother play their part in the secretion and composition of milk. J. R. Slonaker (1931) in studying the effect of different percentage of protein in the diet of nursing rats observed malformations, deformities and blindness in the sucklings due to lack of vitamins in the mammary secretion of the mother, and the addition of yeast to the diet of the mother improved the condition of the sucklings. Kohler, Elvehjam and Hart observed certain growth promoting factors associated with summer milk. They found that rats on summer milk grew more than 4 grams per day while those on winter milk grew only 2½ grams per day. Sure and Schelling found that rats during lactation period required increased amount of vitamin B complex to balance the loss through excretion in the milk. Tarr and McNeile from their study on lactating mothers and infants point

to the greater requirement of the lactating mother for vitamin B complex. Investigations by Strause and MacDonald (1933), Sure and Barnett (1927) have confirmed this observation.

However with regard to infantile cirrhosis it has not been proved conclusively that one must search for the cause of the disease in the pregnant and nursing mothers. The object of the present investigation is to study this question experimentally so that more light may be thrown on the conditions in the maternal organism which may induce changes in the liver of the progeny.

Material and Methods of Study.

Eight albino rats with a total litter of thirty were used for this experiment. Seven nonpregnant rats and seven nursing rats with a total litter of thirty-four were employed as controls. The animals were fed daily with dog-biscuits soaked in diluted milk and oats. Twice a week the diet was supplemented with marmite cabbage and cod liver oil. Two females were housed with a male rate in each cage and vaginal examination for seminal plugs was made every morning. The mated rats were immediately segregated in separate cages.

Of the seven nonpregnant controls six received injections of 0.2 c.c. of pure carbon-tetra-chloride twice weekly, and the seventh was given 0.05 c.c. of carbon-tetra-chloride at the same intervals.

non-pregnant
Carbon-Tetra-Chloride Toxaemia in/control rats.

Animal No.	No. of injections	Dose of each in c.c.	Body wt in gms.	Wt. of liver	Estimated normal wt.	Wt. ratio to normal
B_1	3	0.2	190	6.7	9.5	0.7
B_2	9	0.2	135	7.7	7.5	1.
B_3	8	0.2	175	10	9.05	1.1
B_4	19	0.2	175	11.5	9.05	1.2
B_5	12	0.2	132	8.2	7.4	1.1
B_6	11.	0.2	170	8.	8.9	0.9
A_4	30	0.05	150	9	8.2	1.1

The normal weights of livers have been estimated from chart 3. Hatai. American Journal of Anatomy 15, 1913.

Control Rats with Litter fed on Normal Diet.

Animal No.	Litter No.	Age of Litter in days when sacrificed	Wt, of Litter in Gms.	Wt. of Liver	Estimated Normal wt	Weight ratio Enlargement
	C_1/I	0	4.8	0.25	0.26	1
	C_1/II	0	4.9	0.25	0.27	0.9
C_1	C_1/III	7	11.2	0.5	0.6	0.8
	C_1/IV	7	10.9	0.52	0.57	0.9
	C_1/VI	21	26.3	1.9	2	0.9
	C_1/VII	28	40.8	3	3.05	1
	C_2/I	0	4.8	0.25	0.26	1
	C_2/II	0	4.6	0.25	0.25	1
C_2	C_2/III	7	10.2	0.5	0.5	1
	C_2/IV	14	19.8	1.55	1.52	1
	C_2/V	21	25.6	1.8	1.9	0.9
	C_2/VI	28	43.	2.9	3.15	0.9
	C_3/I	0	5.0	0.26	0.27	1
	C_3/II	0	4.8	0.25	0.26	1
	C_3/III	7	11.1	0.53	0.58	0.9
C_3	C_3/IV	14	20.6	1.5	1.5	1
	C_3/V	21	26.3	1.95	2	0.97
	C_3/VI	28	39.8	2.9	2.95	1

Continued.

Animal No.	Litter	Age of Litter when sacrificed	Wt. of Litter	Wt.of Liver	Estimated Normal wt	Weight ratio Enlargement
C_4	$C_{4/I}$	0	5.3	0.26	0.28	0.9
	$C_{4/II}$	0	4.9	0.26	0.26	1
	$C_{4/III}$	7	11.6	0.6	0.64	0.9
	$C_{4/IV}$	-	10.8	0.51	0.56	0.9
	$C_{4/V}$	15	20.1	1.3	1.5	0.9
C_5	$C_{5/I}$	7	9.6	0.45	0.46	1
	$C_{5/II}$	14	17.8	1.3	1.32	1
	$C_{5/III}$	21	24.8	1.6	1.8	0.9
97	97/I	7	9.2	0.4	0.43	0.9
	97/II	7	9.8	0.46	0.48	1
	97/III	14	19.1	1.23	1.44	0.8
	97/IV	21	27.2	1.8	2	0.9
98	98/I	0	4.8	0.25	0.26	1
	98/II	10	14.	0.9	0.94	1
	98/III	15	17.5	1.3	1.29	1
	98/IV	23	26.8	1.9	2	0.95

The mated controls were allowed to litter and the size of the litter was limited to six by killing off the supernumerary. The young were weighed jointly in a paper bag immediately after birth, and at weekly intervals. The average weight of each was calculated by dividing the total weight by the number of the young weighed. Some of the young were sacrificied soon after birth and at weekly intervals for histological examination of the liver.

In the case of the experimental animals pure carbon-tetra-chloride was injected into the pregnant rat on the back, between the shoulder blades twice weekly by means of a tuberculin syringe fitted with No. 16 needle. The injections were started on different days of gestation for each rat. The first injection was always 0.1 c.c. and the subsequent doses were 0.15 c.c. It was found from previous experiments that injection of more than 0.15 c.c. invariably resulted in resorption, abortion, premature delivery or postnatal death of the litter. After delivery the dose of carbon-tetra-chloride was increased to 0.2 c.c. twice weekly. All the nursing mothers tolerated this dose well. As soon as possible after delivery the newly born litter were jointly weighed and the average weight of each calculated. In some instances one or two newborn were picked out from the litter and sacrificed for histological examination of the liver. In every case the size of the litter was limited to six.

Effect on the litters whose mothers received injections of carbon-tetra-chloride.

Animal No.	Diet Gest	Diet Lact	Date of preg when injec was started	Dose in c.c. Gest	Dose in c.c. Lact	No. of Litter	Age of Litt when sacrificed in days	Wt. of Litter in Gms	Wt of Liver	Estimated normal wt of Liver	Weight ratio to normal
2	Normal	Normal	18th	Total 0.25 c.c	0.2 c.c. twice a week.	2/1	0	5.35	0.25	.28	0.9
						2/II	0	5.7	0.25	.29	0.8
						2/IV	19	27.5	1.83	2	0.9
						2/V	-	25.5	1.62	1.9	0.9
						2/VI	40	52	2.8	3.7	0.8
	Normal	Normal	16th	Total 0.4c.c	0.2 c.c once	3/I	0	4.6	0.23	.25	0.9
						3/II	0	5.1	0.25	.28	0.9
4	Normal	Normal	15th	Total 0.3c.c	0.2 c.c. twice a week	4/IV	24	26.5	1.2	2	0.6
						4/V	24	27	1.4	2	0.7
						4/VI	42	49	3.22	3.5	0.9
						4/VII	42	49.2	2.4	3.55	0.7
9	Normal	Normal	7th	Total 0.75 c.c	0.2 c.c. twice a week	9/I	0	4.6	0.24	.25	1.
						9/II	0	4.5	0.21	.24	0.9
						9/III	28	32.2	1.4	2.4	0.6
						9/IV	28	30.2	1.2	2.3	0.5

Effect on the litters whose mothers received injections of Carbon-tetra-chloride.

Animal No.	Diet		Date of preg when injec was started.	Dose in c.c.		No. of Litter	Age of Litt when sacrificed	Wt. of Litter	Wt. of Liver	Estimated normal wt of Liver	Weight ratio to normal
	Gest	Lact		Gest	Lact						
15	Normal	Normal	19th	Total dose 0.15	0.2 c.c. twice a week.	15/I	0	5.8	0.29	.3	0.9
						15/II	0	5.6	0.27	.29	0.9
						15/III	23	18.2	1.2	1.39	0.9
						15 /IV	23	15.2	1.0	1.07	0.9
28	Normal	Normal	6th	Total dose 0.5 cc.	0.2 c.c. twice a week	28/1	19	17.8	1.1	1.32	0.8
						28/II	19	18.2	1.5	1.36	1.1
						28/III	25	24.4	1.1	1.8	0.6
						28/IV	25	22.5	1.2	1.75	0.7
31	Normal	Normal	16th	Total dose 0.3 cc	0.2 c.c. twice a week	31/I	16	17.8	1.14	1.32	0.9
						31/II	16	16.6	1.15	1.2	1
						31/III	50	72.5	3.8	4.78	0.8
60	Normal	Normal	17	Total Dose 0.4 cc	0.2 c.c. Twice a week	60/V	12	6.8	0.31	0.34	0.9
						60/VI	12	6.2	0.28	0.32	0.9
						60/VII	25	14.3	0.82	.98	0.8
						60/VIII	25	14.5	0.85	1	0.8

The young usually started to feed on the mothers diet from the 18th to the 21st day, but most of them continued to suckle for another week or two. The young were sacrificed from time to time for histological examination, and in most cases the mother was sacrificed a month after delivery.

Many livers were examined in the controls, and in the case of the experimental animals every liver was examined histologically. The tissues were fixed in 10% formol saline and embedded in paraffin. Sections were stained according to requirements with (1) Mayer's acid alum haematoxylin and eosin (2) Heidenhain's Azan for connective tissue (3) Foot and Ménards silver impregnation for reticulum (4) Frozen sections were stained with Sudan III. and counter stained with haematoxylin for fat.

Observation.

The general effect on the nonpregnant adult rats which received 0.2 c.c. of carbon-tetra-chloride twice weekly was a rapid decline in the body weight in all animals. Most of them took less than half the amount of their usual requirement of food after two injections. The animals which were receiving the carbon-tetra-chloride in 0.2.c. doses began to die one after the other. Five of these died within 45 days from the beginning of the experiment, and one was sacrificed after the 19th injection. The animal which was receiving 0.05 c.c. died after 4

months. Ascitis was present in two, and histologically all the livers showed varying degrees of liver damage and fibrous tissue proliferation depending on the dosage of carbon-tetra-chloride.

Histology of the liver of rats which had 3 injections of 0.2 c.c. of carbon-tetra-chloride.

There was necrosis of liver cells around the radicles of the hepatic vein which extended outwards to coalesce with necrotic areas in the neighbouring lobules. There was no cellular infiltration of the necrotic debris, The sinusoids in the centre of the lobule had ruptured and numerous red cells were observed in the debris of cells. The peripheral and midzonal regions were only slightly affected, and the parenchymal cells showed cloudy swelling and early fatty change. A few bi-nucleate liver cells, and cells undergoing mitosis were present. There was marked congestion affecting the whole vascular system; but the structures in the Glisson's Sheath were unaffected.

In the liver of the rat which had 8 injections of 0.2 c.c. all the parenchymal cells in the centre of the lobule showed coagulation necrosis. The cell outline was hardly visible and the nuclei were in various stages of Karyorrhexis and lysis. There was marked infiltration of the necrotic debris with histocytes, fibroblasts and a few polymorphs. The wall of the hepatic radicles appeared oedematous, and slightly thickened. The cells throughout the lobule were

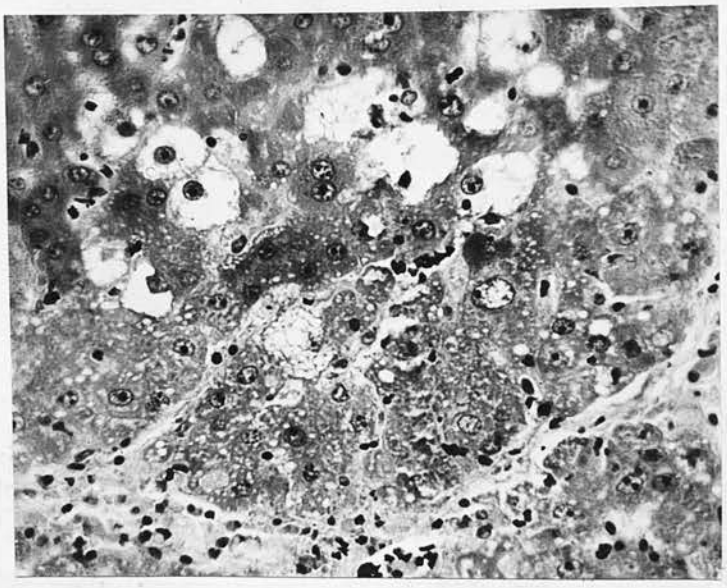


Fig. I. Liver of control rat B_3 which had received 8 injections of 0.2 c.c. of carbon-tetra-chloride. Necrosis of liver cells radiating outward from the centre is observed. The debris is infiltrated with polymorphs histiocytes and fibroblasts. The liver cells in the middle zone show hydropic degeneration and fatty change.

H and E x 300.

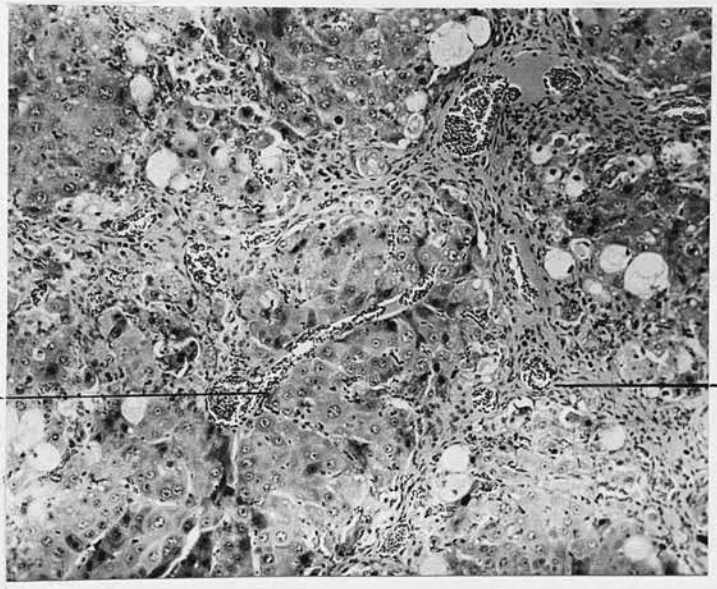


Fig. II. Liver of control rat B_4 which received 19 injections of 0.2 c.c. of carbon-tetra-chloride Mono-lobular cirrhosis with fibrous tissue more central in distribution.
P. portal tract which is scarcely affected.
C. collecting vein.

H and E x 85.

affected to a greater or lesser extent by degenerative changes. A large number of these were hydropic, and the cytoplasm of the other cells was vesicular, and the cell outline indistinct, as if the cells had fused together. The portal tract and a narrow fringe of cells abutting against it were healthy.

In the case of the rat which had 19 injections of 0.2 c.c. the liver showed bands of fully formed highly cellular fibrous tissue extending between the radicles of the hepatic vein and the portal sheath, thus breaking up the normal lobular pattern to give it the appearance of mono-lobular cirrhosis. The amount of fibrous tissue around the hepatic venous radicle was found to be definitely greater than that found in the Glissons Sheath. Occasionally the branches of the hepatic vein showed some degree of periphlebitis and thickening of the wall. A few portal tracts were practically unaffected, and there was no increase of fibrous tissue in them. Bile duct proliferation was slight. The parenchyma in the centre of the pseudo-lobules appeared healthy and those cells which were in close proximity to the portal tracts were deeply stained, and their nuclei contained more chromatin. Many of these were bi-nucleated. Towards the periphery of the pseudolobules the cells were markedly hydropic with pyknotic nuclei in the centre of the cells. The histological picture resembled rather closely the toxic cirrhosis described by Mallory (1911) with necrosis of cells around the hepatic

Fig III. Liver of control rat B_4. The portal tract with the hepatic artery, portal vein and bile duct are practically unaffected by the fibrosis. The adjacent parenchymal cells show deeper stain in the cytoplasm and intensely basophilic nuclei.

H and E x 350.

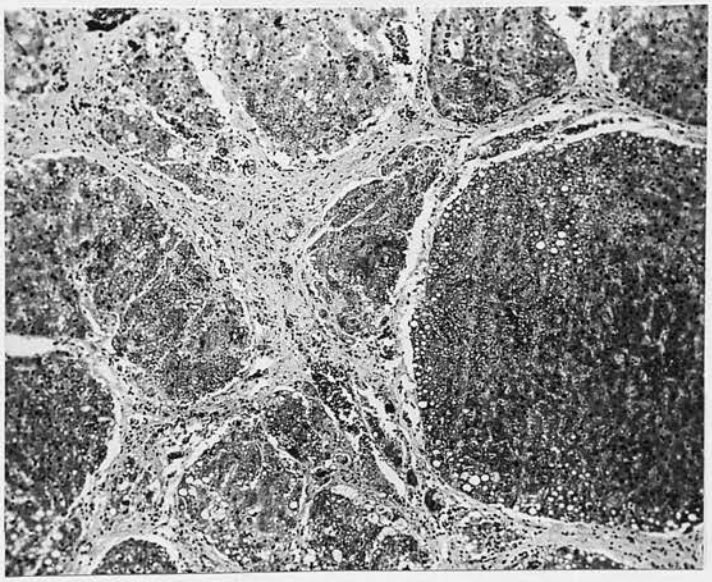


Fig IV. Liver of control animal A_4 which received 30 injections of 0.05 c.c. of carbon-tetrachloride. Portal as well as hepatic veins are surrounded by bands of fibrous tissue which also encircle large and small islands of regenerated nodules.

H and E x 75.

veins which later lead to central fibrosis.

In the liver of the rat which had 30 injections of 0.05 c.c. of carbon-tetra-chloride twice weekly, narrow columns of fibrous tissue were seen to encircle islands of hepatic parenchyma varying in size from 0.5 m.m. to 5 m m in diamater. The cells were vesicular, and frozen sections stained with Sudan III. showed minute globules of fat in the cytoplasm. The cell outline was indistinct and many of the nuclei were swollen, besophilic and opaque. Here and there extreme fatty degeneration affecting whole masses of cells in some of the smaller islands of parenchyma, could be seen. There was marked infiltration with lymphocytes, plasma cells, and histiocytes in the fibrous tracks. The blood vessels did not show any appreciable change. Proliferation of bile ducts was scanty. The appearance was typical of Laennecs or multilobular cirrhosis.

Histology of the liver in the newborn control.

The hepatic cells were arranged in anastomosing cords between which could be seen the profuse intralobular sinusoidal circulation. The parenchymal cells were irregularly polygonal in shape and differentiation between individual cells was more obvious than in the adult liver. The cytoplasm was faintly basophilic. The chromatin of the nucleus was irregular in distribution, and the nucleolus was prominent, staining a deep violet colour. A few cells were binucleated.

The blood vessels were thin walled with wide lumen. Numerous foci of blood cell proliferation and differentiation were present. The haemopoetic foci were chiefly extra vascular in distribution apparently differentiating from the mesenchyme of the sinusoids. Intra sinusoidal haemopoesis was also evident in some places. Gradations of cells ranging from basophilic erythroblasts to acidophilic normoblasts with deep staining nuclei could be made out in the haemopoetic foci. Megakaryocytes having several nuclei were observed frequently. The cytoplasm of these cells was more basophilic than the liver cells and the nucleolei more prominent.

The architectural pattern of the liver in a week old rat was well formed. The cells resembled more the adult type; but were relatively smaller in size. Connective tissue of the portal tract was sparse, and the vessels were thin walled. In the liver of most of the young a few globules of fat in the cells of the central zone of the lobules could be made out. The foci of haemopoetic cells had mostly disappeared; but a few isolated clumps of these cells were occasionally present.

In the liver of the two weeks old animal the haemopoetic cells had complete disappeared and the structure of the liver resembled the adult. In most livers there was no trace of fat in the centre of the lobules.

During the third and fourth weeks the liver was identical with that found in the adult, except that the lobules were slightly smaller in size.

Effect on the litters whose mothers received injections of carbon-tetra-chloride in doses of 0.15cc twice weekly during the latter period of gestation and 0.2 c.c. twice weekly during lactation.

General effects. No remarkable difference between the average weight of the newborn experimental litter and control litter was revealed. In most animals the average weight per litter seemed to depend to a large extent on the size of the litter; but there was a marked lag in the growth of the experimental litter as compared with the control. No runts were observed in any of the litter; but some gained weight much more gradually than others. The young ones took a longer period to be weaned completely; and the gain in weight was more rapid after they were weaned; but it never equalled that of the control animals.

Histology of the liver of the newborn. The parenchymal cells were swollen and vesicular. The cytoplasm failed to take the stain except for a narrow fringe around the nucleus and the cell margin. The nuclei were ill stained, distorted and in many the nucleoli could not be differentiated from the irregular masses of nuclear chromatin. The islands of haemopoesis seemed to have escaped injury. The wide

Fig. V. Liver of control rat A_4 showing development of coarse cirrhosis after 30 injections of 0.5 c.c. of carbon-tetra-chloride.

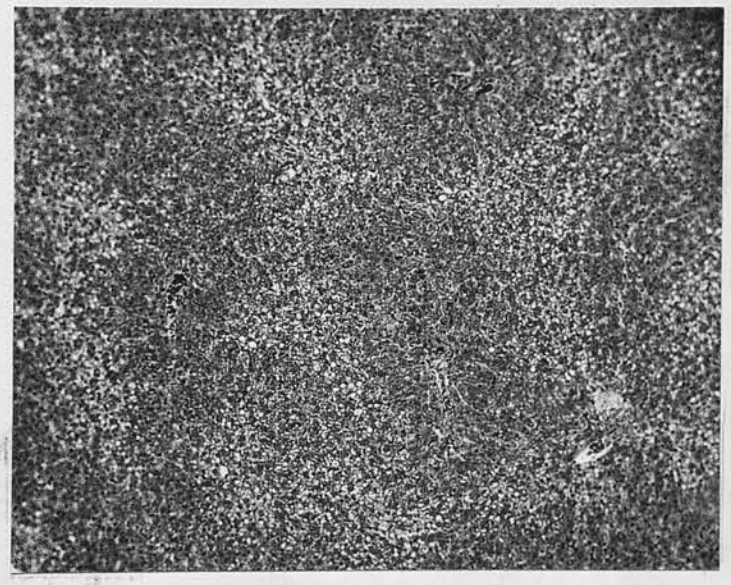


Fig VI. Liver of litter $4_{/IV}$ showing moderate fatty change in the parenchymal cell around the central vein.

anastomosing channels of sinusoids that are normally present could not be made out in the whole section as they were collapsed through swelling of the parnechymal cells. A few distorted red cells were observed between the cords. There was moderate distension of the hepatic and portal vessels.

The liver of the young in the second week of life showed marked fatty degeneration of the parenchymal cells throughout the lobule; but it was more intense around the central veins in each lobule. In some livers the cell membranes were indistinct and only the nuclei seemed to be left intact. Necrosis of a few cells in the centre of the lobule was frequently seen, and such areas were the seat of round cell and histiocytic infiltration. In one liver 28/I bile thrombi were seen in the hepatic cords, and many of the Kupffer cells were impregnated with bile pigment. A few cells near the portal tract showed mitosis. The wall of the hepatic veins and their radicles were relaxed in most cases. The patency of the sinusoids differed in each case. In some they were dilated and in others collapsed. There was a slight proliferation of the Kupffer cells in all the livers. The Glisson's Sheath and the structures in it were usually healthy; so also was the peripheral zone of parenchymal cells adjoining the tracks.

Third week. There was slight proliferation of Kupffer cells throughout the lobule, and they appeared more

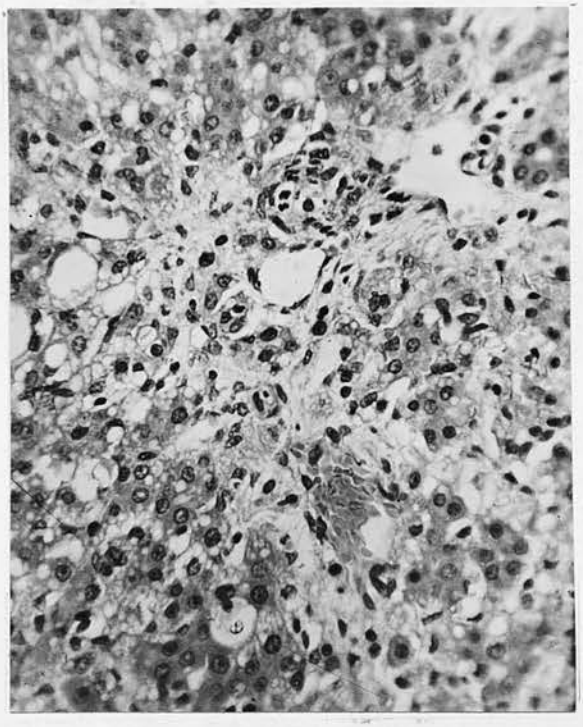


Fig. VII. Liver of litter 28/I showing necrosis of a few cells around the central vein and infiltration with histocytes. The remaining liver cells showmoderate fatty change. H and E x 350.

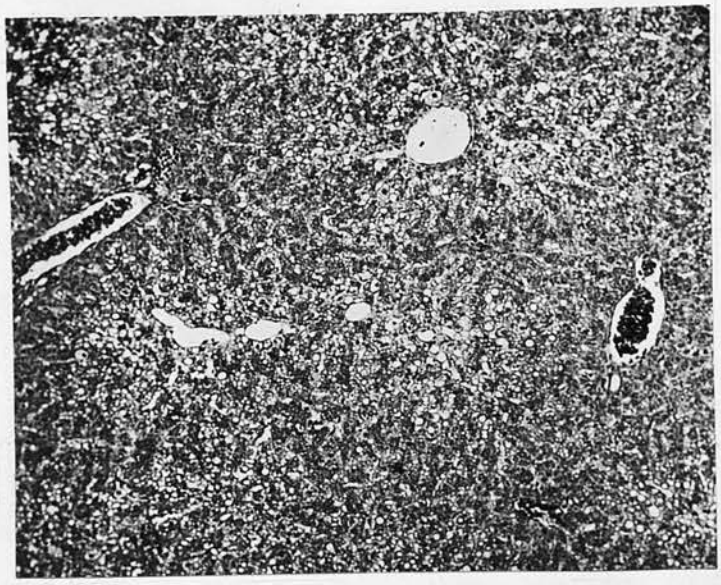


Fig VIII. A. Liver of 31/II showing wide-spread fatty change with indistinct cell outline. Many of the nuclei towards the centre of the lobule are pyknotic. The blood vessels are relaxed. H and E x 80.

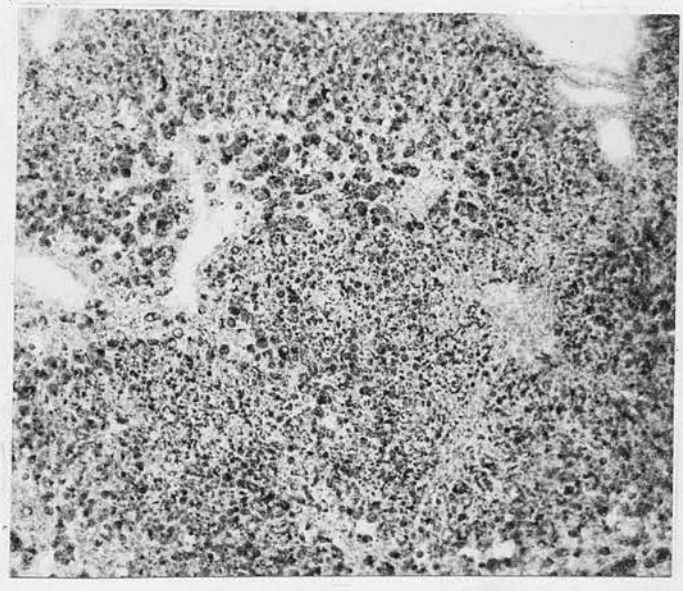


Fig. VIII. B. Liver of 31/II. See coloured plate. Frozen section stained with Sudan III. x 80.

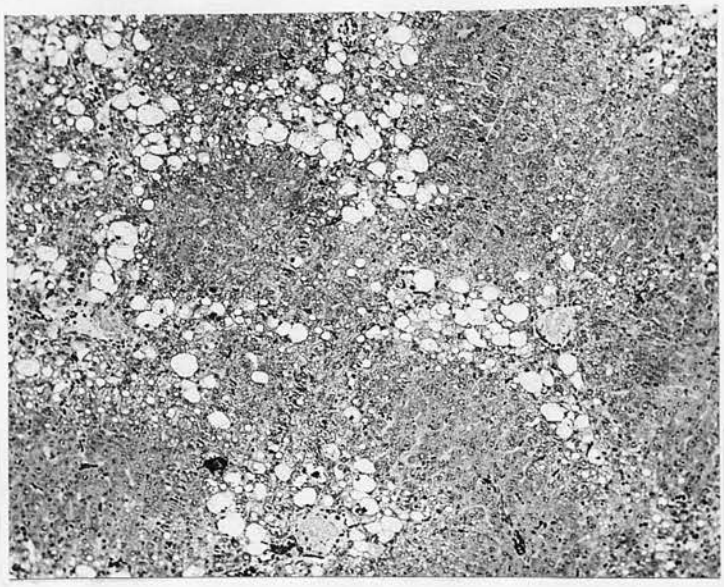


Fig. IX. Liver of litter 15/IV whose mother was receiving injections of 0.2 c.c. carbon-tetra-chloride twice weekly. There is necrosis and marked hydropic degeneration of liver cells at the centre of the lobule.
H and E x 90.

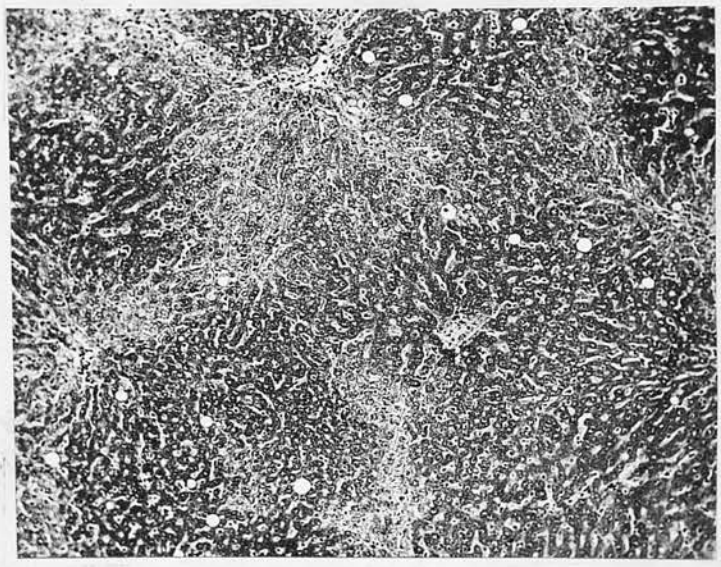


Fig. X. Liver of rat 15 which had been receiving 0.2 c.c. of Carbon-tetra-chloride twice weekly. Slight fatty change is seen in the centre of the lobule, but when compared to the degenerative changes in the liver of the litter it is insignificant.

H and E x 90.

prominent than usual. A few foci of central necrosis, and round cell infiltration were seen in some of the livers; but on the whole fatty degeneration was the characteristic change at this age. In the livers of 15/III. AND 15/IV., there was hydropic dengeration of a zone of cells in the centre of the lobule, which had a tendency to spread out towards the neighbouring lobules. The cells in the centre of the lobule were in every instance affected to a greater extent than those at the periphery; the portal vein, the artery, the duct and the cells in the immediate vicinity of these structures were usually spared.

Fourth week. After 21 days the changes in the liver were found to diminish progressively, as the litter began to feed on the diet allowed for the mother. Moderate fatty change was still to be seen around the central vein, and frequently there was generalized hyperaemia of the whole lobule with distension of the blood vessels. By the end of the fourth week the liver in most of the litter assumed a normal appearance.

Effect on the parent rat.

It was observed in the experiment on the effect of toxic doses of carbon-tetra-chloride during gestation that 0.2 c.c. of the drug was not tolerated by the pregnant rat. In the present series of experiments it was seen that the animals tolerated the reduced dosage of darbon-tetra-chloride well during

gestation. During lactation a dose of 0.2 c.c. did not have any remarkably injurious effect on the parent; except, that the animal gradually declined in weight. There was no death in any of the mother rats. They took their feed just as well as the control animals.

Histology of the Liver. The livers of all the experimental mothers showed varying degrees of fatty degeneration affecting the cells throughout the lobule. In the majority of animals there was no necrosis of liver cells, and if at all there was any, it was usually limited to a narrow zone around the central vein. This area of necrosis never extended further outwards. Frequently there was slight infiltration with histiocytes in the centre of the lobule. In no case was there necrosis sufficient in extent to resemble that seen in the control animals receiving the same dose of carbon-tetra-chloride.

SUMMARY.

(1) The effect on the suckling rats whose mother had been receiving injections of a cirrhogenic toxin during the later period of gestation and lactation was to produce stunting effect on growth and to induce degenerative and sometimes necrotic changes in the liver.

(2) The liver changes became progressively less in extent as the young ones were weaned.

(3) Repeated toxic doses (0.2 c.c.) of Carbon-tetra-chloride which frequently resulted in the death of the control animals was tolerated well by nursing mothers.

(4) The same dose of Carbon-tetra-chloride which resulted in severe necrotic lesions in the liver of the control animals produced only a slight damage in the liver of the nursing mother.

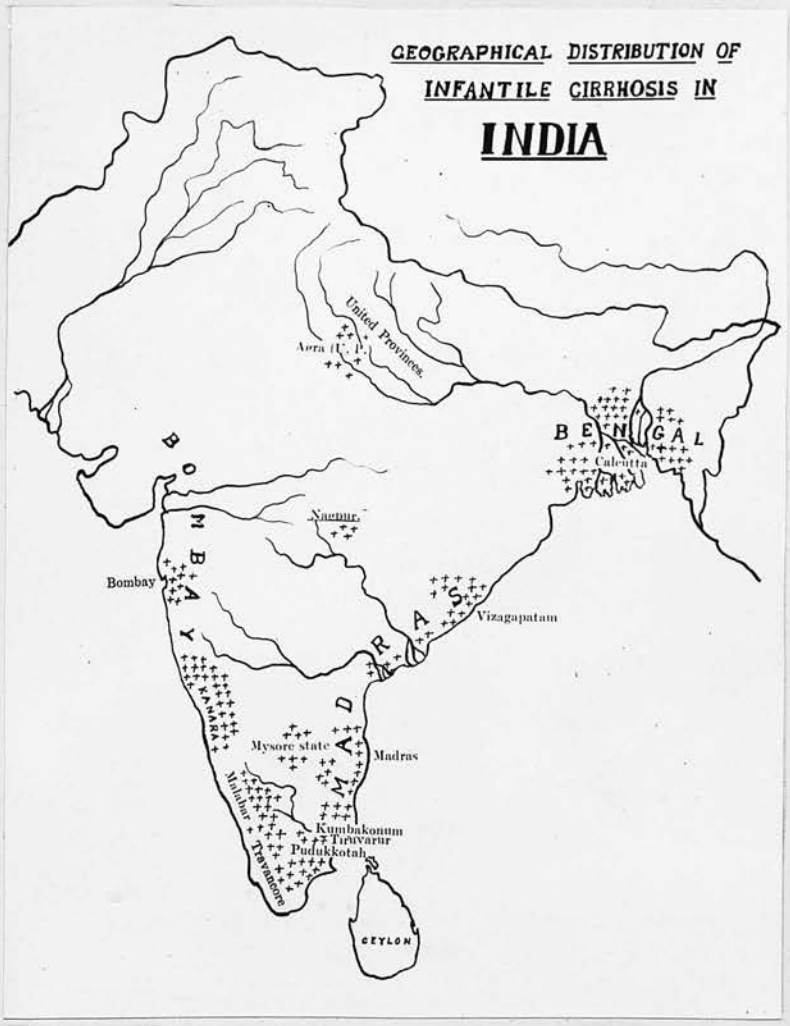
(5) It was seen from the histological study of the changes produced in the liver of nonpregnant rats with repeated toxic and repeated small doses that the former induced a toxic cirrhosis with fibrosis central in distribution whereas the latter produced a multi-lobular or Laennec's cirrhosis.

The relation between infantile cirrhosis and a diet of polished rice.

Experimental production of liver damage in suckling rats whose parent is fed on polished rice and vitamins and is subjected at the same time to the action of cirrhogenic toxin.

I N T R O D U C T I O N.

Geographical distribution of the disease. The peculiar geographical distribution of the disease was a feature which attracted the attention of the earliest writers on this subject. (Sen (1882) a pioneer in the study on "infantile cirrhosis" first observed its prevalence in Calcutta in contrast to its absence in Central and North West provinces. Ghose J.W. (1894) and Gibbons (1891) recorded their cases from Calcutta and Lower Bengal. Mackenzie recorded cases of infantile cirrhosis in Kanara and South-West Coast of Bombay noting at the same time its scarcity in Bombay, Sind Gugarat, Aden, and Deccan. Pearce T. F. included Madras also as an endemic area, and Singh recorded cases from United Provinces as well. Castellani and Chalmers (1919), Byam and Archibald (1922) noted the prevalence of the disease in Calcutta chiefly. De Costa reported a case from Nagpur. Vaidyanath Iyer (1926) reported cases from Tellicherry on the West coast of India and Krishna Iyer (1926) came across the disease in the Tamil districts and in the eastern districts of South India. Manson-Bahr (1927)



Map of India.

The geographical distribution of Infantile cirrhosis in India is marked out from the data gathered from medical literature.

The disease is seen along the sea coast and in the deltas of the great rivers where rice forms the chief cultivation. In the centre of the peninsula and in the North Western provinces where no rice is grown the disease does not occur.

described the disease as occurring in Bengal, Madras, United Provinces and Bombay. Mukherji (1927) in his attempt to study the geographical distribution of "infantile cirrhosis" has left a valuable record of the endemic areas for this disease. According to him, they are Calcutta, and various districts of Bengal, Madras, and the districts Arcot, Salem, Trichinopoly, Madura, Ramnad, Tanjore, Pudukota in the Madras presidency, a few cases from Mysore, and some from United provinces and Bombay. Sankara Iyer (1931) has reported some cases from Mysore, and Pandalai has included Travencore in the endemic area along with Calcutta, Bombay, Madras United Provinces, Malabar and Mysore. Phillipy Rezek from his study of the distribution of the disease found that it was only found in Bengal, the surroundings of Madras, South India and Vizagapatam. On considering the incidence of the disease in its relation to the diet in different parts of India we arrive at the definite connection between it and the rice eating population of India. It is estimated that over a third of the population in India live on rice. However if we exclude the deltas of the great rivers and the narrow coasted strip rice is a rare crop throughout the rest of the peninsula. It is exclusively the staple diet where it is grown. The out turn of rice is greatest in Bengal and Assam. A large percentage of the acreage is under rice cultivation in the deltas of Godavari, Kistna, and Canvery,

in the lowlands of Travencore, Malabar, and Kanara. In the North Western Provinces it is grown only in the damp localities and form the favourite food for upper classes. In Madras the area under rice is about 33% of the food growing area, in Bombay it is 10%, in Sind it is 17%, in the Central Provinces 34% and in Punjab 5%. In the $\frac{2}{5}$ of India under Indian Princes, which cover the centre of the peninsula scarcely any rice is grown. It will be seen from a study of the literature that the incidence of infantile cirrhosis is greatest in Bengal and Madras provinces, in the deltas of the great rivers, and along the long strip of land fringing the coast. Bhaskara Menon and Annamalai from their study of the incidence of hepatic cirrhosis in South India find a high incidence of death from cirrhosis in Madras. They also report that the incidence of cirrhosis is 6.9% in 489 autoposies in Calcutta, 9.3% in 535 autopsies in Vizagapatam, whereas Berlin figures indicate only 1% in 3,200 autopsies. In Java where the population live almost exclusively on rice mortality from cirrhosis is 10 times that seen in an European population of the same age and sex in Holland.

INDIAN FOOD-GRAINS.

Province	Percentage of Food-Grain area under			Total Population	Population eating rice
	Wheat or Barley	Millets	Rice		
Punjab	54	41	5	20	1
N.W.Provinces	57	34	9	42	4
Bengal and Assam	not known		-	66	46
C.Provinces	27	39	34	8	3
Berar	17	82	1	2	--
Bombay	7	83	10	17	2
Madras	--	67	33	31	10
Mysore	--	84	16	5	1

Figures taken from "The Indian Empire"
by W. W. Hunter.

Ethnological and Social considerations.

Reports of cases of infantile cirrhosis from whatever source it may be always agree on one point and that is the incidence of the disease exclusively among the rich upper and middle class families. On analysing the diet of various classes of people and the market value of the different varieties of grains available in an area for consumption it will be seen that, that it is the wealthy upper and middle class families who live almost exclusively on rice in the rice growing districts. The whiteness of the polished

rice is preferred by them to the coarser but more nourishing unpolished or parboiled rice.

The poor people who form the bulk of the population live on the different varieties of millet that are grown in all parts of India. The lower middle class whose staple diet is rice in the rice growing areas, invariably live on parboiled unpolished rice. Infantile cirrhosis is rarely if ever seen in these families and in the poor who live on millets.

Dietetic Habits. Ever since the disease attracted the notice of the medical profession it was observed that the incidence of the disease was much greater among the Hindus than among the Mohammedans, Anglo Indians, Indian Christians or Europeans. The health officers report of mortality in infants in Calcutta due to this disease shows a marked disproportion between the death rate for Hindu and Mohammedan children, despite the fact that the population of Hindus and Mohemmedans are about equal in Calcutta.

Death rate due to infantile cirrhosis in Calcutta according to the Health Officers report for the three years, 1891 to 1893.

Year	Hindus	Mohammedans.
1891	486	21
1892	546	30
1893	584	29

In Madras the disease is seen more frequently among the Brahmin Community than in the rest of the population put together, even though the Brahmin Community form only a small percentage of the population. In a "preliminary report on the prevalence of Infantile biliary cirrhosis in the Mysore State" ~~by~~ Sankara Iyer reports that out of 60 cases seen by him 90% were among Brahmins. The reason is not far to seek as it is well known that Mohammedans, Indian Christians Anglo Indian and Europeans live on a mixed diet containing a large proportion of animal protein whereas many communities among Hindus are strict vegetarians. It is also a fact that in many Hindu families where the husband may be a meat eater, the wife is a strict vegetarian because of religious scruples. In South India especially the Brahmin Community as a whole never take meat, fish or egg in any form, and a large body of Non-brahmin Hindus are also strict vegetarians. J. N. Ghose (1894) observed that Mohammedans and Eurasians feed their children occasionally with animal broth whereas the Hindu mother always nourished her children from her breast. Tirumurti and Radhakrishna Rao and others have commented on the incidence of the disease among vegetarians.

Another factor which may be of some significance in considering the etiology of the disease is that before the year 1874 the disease was unknown. J.W. Ghose (1894) called it a new disease as it had not been treated in the Medical books of his time. It is more than probable that

the appearance of the disease coincides with the date from which highly milled rice became popular among rice consumers in towns.

Nakahara, Fijiwara, and Mori in their study on Cancer development in the liver have noted a certain geographical similarity in the occurrence of beri-beri and hepatoma in man. Though there is a similar geographical distribution there has been no report of a greater incidence of cirrhosis among sufferers from Beri-beri; nor did any of these cases of 'infantile cirrhosis' show symptoms of beri-beri. What exactly was the principle that was lacking in the vegetarian diet of polished rice which while not lacking in vitamin B complex yet helped to bring about liver damage was the question that required a solution. A perusal of the work of Paul Gyorgy and Harry Goldblatt on the production of liver necrosis in rats on a nutritional basis shed some light on the subject. They found that in rats placed on a diet that was adequate in all essentials, but was wanting in yeast eludate (yeast extract) developed extensive necrosis of the liver and sometimes cirrhosis. Neale and H. C. Winten (1938) in their experiments on rats found that soxium x-anthine and other purine substances, exerted a protective action on the liver against carbon-tetra-chloride toxaemia.

During the process of milling and polishing the rice, it is the germ or embryo that is first to be removed. It is this germ that is rich in nucleic acid

and purine substances in addition to the vitamin B complex. Wheat germ has often been used as a rich source from which to extract nucleic acid. The germ of the rice is also rich in the supply of nucleic acid. Bearing these facts in mind the present experiment on the effect on the offspring from the administration of carbon-tetra-chloride to the pregnant and nursing mothers who is fed on a basic diet of polished rice was carried out.

Material and methods of study.

The principle difficulty in choosing a diet consisting mainly of polished rice was the supply of essential vitamins without including in the diet at the same time the antinecrotic factor. It was found that pregnant rats frequently aborted, and that postnatal mortality of the litter was high in test animals that were fed only on polished rice, vitamin B_1, wheat germ oil, and cod liver oil. As a result it was decided that the experiment could best be carried out with a normal diet during pregnancy and with a special diet during lactation. It was impossible because of the war to obtain either riboflavine or vitamin B_6 from any of the chemical firms in Great Britain, and the difficulty was partly solved by feeding the animals on polished rice with wheat germ oil, cod liver oil, vitamin B_1 and adding just enough yeast to keep the litter alive. It was found that unless a certain

minimum of yeast was added to the diet of the nursing mother the litter did not survive for more than a week.

The basic diet in the experiment consisted of:-

Polished rice	10 grams.
Sodium chloride	0.1 grams
cod liver oil	2 minims.
Wheatgerm oil	0.5 minims.
Vitamin B_1	10 international units.

(The vitamin B_I was obtained in the form of 'Benerva' tablets. 'Roche'. One tablet was dissolved in 33.3 c.c. of distilled water so that one c.c. contained 10 international units of B_I)

To this varying amounts of yeast and sometimes meat and casein were added, depending on the experiment.

Preparation of the diet. The polished rice was washed well three times in different changes of water, and boiled with five times its bulk of water for 20 mins. The excess of water was strained off. When cold, Sodium chloride, cod-liver oil, wheat-germ oil, and vitamin B_I were added and mixed well. Rice takes up about three times its weight of water when boiled, and the feed was always kept much in excess of the rats requirement. It was found by weighing out what was left each day and after making due allowance for wastage that a rat consumed per day on an average an amount of boiled rice equivalent to 10 grams of uncooked rice. On the protocol of these experiments

the amount in grams of yeast noted is for a diet equal in value to 10 grams of uncooked rice.

Control. Two rats No. 37 and No. 45 were used as controls. They were fed during gestation on normal diet and during lactation on the basic diet with the addition of 1 gram and 2.5 grams of yeast respectively. The young were sacrificed at intervals and the livers were examined histologically.

Experiment. Twelve albino rats with a total litter of 48 young were employed in this experiment. The date of copulation was noted for each animal through daily examination for vaginal plugs. The mated animals were segrated immediately in separate cages. In most cases the injections were started after the 12th day of gestation. In every case the first dose was 0.1 c.c. and the subsequent doses were 0.15 c.c. of carbon-tetra-chloride during the period of gestation. The expectant mothers were inspected twice daily so that the number of young in the litter may be checked soon after delivery. The size of the litter in every case was limited to six by killing off the supernumerary. The same day the nursing mother was put on the special diet.

Three animals Nos. 19, 20 and 30 were fed on the basic diet supplemented with 2 to 2.5 grams of yeast; but in the case of No. 20 the yeast was discontinued from the 10th day of lactation.

Control Animals on Special Diet.

Animal No.	Diet		No. of litter	Age of Litter when killed in days.	Wt. of Litter in Gms.	Wt. of Liver	Estimated normal Wt.	Wt. ratio to normal.
	Gest	Lact						
37	Normal	Basic Diet +1gm yeast	37/I	20	10	0.41	0.48	0.9
			37/II	20	9.8	0.4	0.47	0.9
			37/II	36	14.3	0.65	0.98	0.7
45	Normal	Basic Diet +2.5 gm yeast	45/I	13	12.5	0.42	0.76	0.6
			45/II	13	11.5	0.4	0.62	0.6
			45/III	23	22.2	1.5	1.6	0.9
			45/IV	30	25.8	1.7	1.8	0.9

The normal weights of livers have been estimated from Table 1. Hatai. American Journal of Anatomy 15. 1913.

Experimental production of liver damage in suckling rats whose parent is fed on polished rice and vitamins, and is subjected at the same time to the action of carbon-tetra-chloride.

Animal No.	Diet		Day of gest when injec was started	Dose In c.c.		No. of Litter	Age of Litt when sacrificed in days	Wt of Litter in Gms.	Wt. of Liver	Estimated normal wt of Liver	Weight ratio to normal
	Gest	Lact		Gest	Lact						
11	Normal	Basic Diet + 1 gm Yeast	19	0.2 c.c. Total dose	0.2 c.c. twice a week	11/I	0	4.5	0.21	0.24	0.9
						11/II	0	5.2	0.25	0.28	0.9
						11/III	28	20.05	1.15	1.5	0.8
						11/IV	28	19.04	1.02	1.44	0.7
						11/V	40	27.2	1.7	2.	0.8
						11/VI	40	22	1.2	1.6	0.7
17	Normal	Basic diet + 1 gm yeast	3	0.6 c.c. Total dose	0.2 c.c. twice a week	17/1	24	10.2	0.7	0.5	1.4
						17/II	24	12.4	0.8	0.74	1.1
						17/III	29	12	0.65	0.7	0.9
						17/IV	29	11.2	0.6	0.6	1
						17/V	30	10.2	0.75	0.5	1.5
						17/VI	30	11.5	0.8	0.62	1.3
18	Normal	Basic Diet + ½ gm marmite thrice weekly	13	0.3 cc Total dose	0.2cc twice wkly	18/I	8	6.9	0.32	0.35	0.9
						18/IV	22	10.4	0.8	0.52	1.6
						18/V	22	11.6	0.84	0.64	1.3
						18/VI	29	15.5	1.6	1.1	1.4
						18/VII	29	14.	1.4	0.94	0.1
19	Normal	Basic Diet + 2 gm yeast	11	0.4 cc Total dose	0.2cc Twice a wk.	19/I	21	19.5	1.1	1.5	.7
						19/II	27	20.2	1.9	1.5	1.3

Continued.

Animal No.	Diet Gest	Diet Lact	Day of gest when injec was started.	Dose In c.c. Gest	Dose In c.c. Lact	No. of Litter	Age of Litt when sacrificed	Wt. of Litter	Wt. of Liver	Estimated normal wt of Liver	Weight ratio to normal.
20	Normal	Basic diet + 2 gm yeast for 10 days	11	0.45 c.c. Total dose	0.2 c.c. twice wkly	20/I	23	8.1	0.6	.39	1.5
						20/II	23	6.5	0.5	.34	1.4
						20/III	23	6.3	0.55	.33	1.6
21	Normal	Basic diet + 1 gm yeast	12	0.4 c.c. total dose	0.2 c.c twice wkly	21/I	0	4.2	0.22	.24	.9
						21/II	0	3.6	0.2	.2	1
30	Normal	Basic Diet + 2.5 gm. Yeast	15	0.45 c.c. Total Dose	0.2 c.c. twice wkly	30/I	17	11.8	0.52	.66	.8
						30/II	17	11.7	0.52	.64	.8
						30/III	24	12.5	0.51	.75	.7
						30/IV	24	19.8	0.45	.56	.8
						30/V	31	17.5	1.15	.129	.9
						30/VI	31	16.5	1.1	1.19	.9
33	Normal	Basic Diet + 3 gm meat per day for 16 days	15	0.3 c.c. Total Dose	0.2. c.c twice wkly.	33/I	16	10.5	0.6	.53	1.1
						33/II	16	11.8	0.62	.66	.9
						33/III	23	10.8	0.7	.56	1.3
						33/IV	23	10.2	0.68	.5	1.3
						33/VI	34	12.	0.65	.68	.9
39	Normal	Basic Diet + 1 gm. yeast	14	0.4 c.c total dose	0.2 c.c twice wkly	39/I	19	5.5	0.3	.29	1.
						39/II	26	7.8	0.7	.38	1.8

Continued.

Animal No.	Diet Gest	Diet Lact	Day of gest when inject was started.	Dose Gest	Dose In c.c. Lact	No. of Litter	Age of Litt when sacrificed	Wt. of Litter	Wt. of Liver	Estimated normal wt of Liver	Weight ratio to normal.
44	Normal	Basic Diet + 1 gm. yeats	11	0.4 c.c. total dose	0.2 c.c. twice wkly	44/I	15	4.4	0.25	.24	1.
79	Normal	Basic Diet + 3 Gm meat	10th day	0.4 c.c. Total dose	0.2 c.c. twice a wk	79/I	8 days	13.6	0.5	.9	.6
						79/II	8 days	13.2	0.47	.85	.6
						79/III	24 days	30.8	1.9	2.3	.8
						79/IV	24 days	31.2	2.0	2.4	.8
83	Normal	Basic Diet + 3 gm Casein	14th day	0.4 c.c. Total dose	0.2 c.c. twice a week	83/I	6 Days	10.3	0.45	.07	.8
						83/II	6 days	10.5	0.48	.52	.9
						83/III	15 days	12.3	0.52	.74	.7
						83/IV	15 days	12.6	0.51	.76	.7
						83/V	22 days	21.5	1.35	1.65	.8
						83/VI	22 days	21.2	1.2	1.6	.7

Five animals were fed on the basic diet and 1 gram of yeast. The animals were Nos. 11, 17, 21, 39, 44.

No. 18 was fed on the basic diet with the addition of 0.5 grams of marmite thrice weekly.

Two animals Nos. 33 and 79 were fed on the basic diet with 3 grams of meat; but the meat was discontinued for the animal No. 33 after the 16th day.

One animal No. 83 was put on the basic diet with 3 grams of casein.

During lactation each animal was injected with 0.2 c.c. of carbon-tetra-chloride twice a week subcutaneously between the shoulder blades. Most of the litter were weighed soon after delivery and at intervals to keep a record of the rate of growth. The young were sacrificed from time to time and the livers were fixed in 10% formol-saline for histological examination.

Observations.

Effect on control litters of Nos. 37 and 45.

There was a definite retardation in the growth of the litter especially in the case of litter No. 37 whose mother was fed on the basic diet with 1 gram of yeast. In the case of No. 45 the rate of growth did not lag far behind those of the control rats on normal diet. In all the young the stomach contained curdled milk, and the stunting of growth was apparently

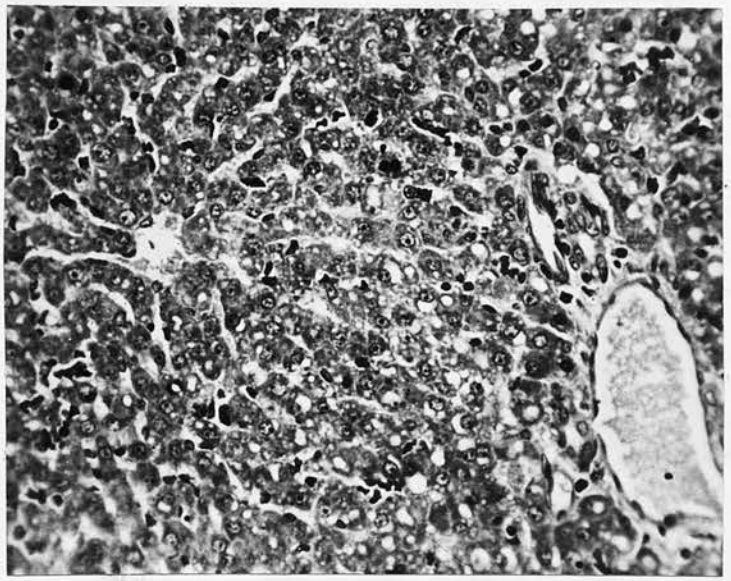


Fig. XI. Liver of control litter aged 18 days showing a hepatic and a portal terminal. The cells are well preserved but there is a slight infiltration of fat. The cells in the centre as well as in the periphery of the lobules are well stained.
H and E x 350.

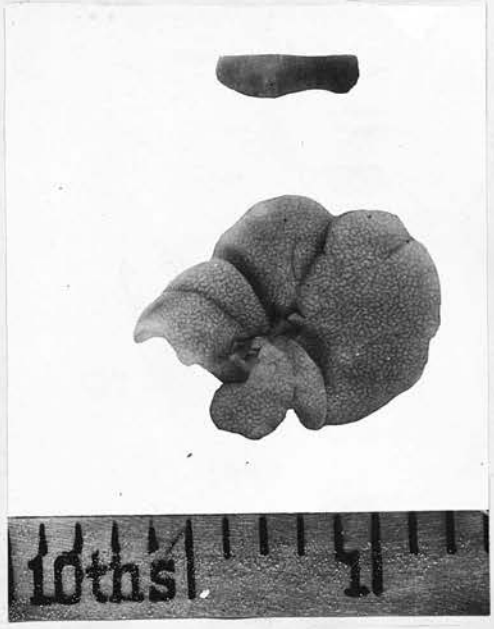


Fig. XII. Liver of litter 17/IV aged 29 days whose mother had been receiving injections of Carbon-tetra-chloride. Note the exaggeration of the lobular pattern and the finely granular surface.

not due to starvation.

Histologically. The livers of the young did not exhibit much variation from those of the controls whose mothers were on normal diet. However in many livers the parenchymal cells were highly vesicular with granular cytoplasm; and in a few there was a greater amount of fat than is normally found in the liver of the young. No serious liver damage was observed in any of the livers.

Effect on the litter of the experimental animal

(1) In the litters Nos. 19, 20 and 30 whose mothers were fed on the basic diet supplemented with 2 to 2.5 grams of yeast there was moderate retardation in the average gain in weight of the litter; This lag was most marked in the litter of the rat No. 20 in whose diet the supplement of yeast was discontinued from the 10th day.

In the sucklings the liver changes were striking during the third and fourth week. There was extensive hydropic degeneration and fatty changes in most of the liver cells leaving only islands of healthy liver cells at the periphery of the lobule in the neighbourhood of the Glisson's Sheath. The cells showing hydropic degeneration were ballooned out to a great size. The nuclei were pyknotic and the cytoplasm scanty and granular. In some of these cells there was no stainable cytoplasm and the nuclei had disappeared. There was slight lymphocytic and histiocytic infiltration in the centre of the lobule where degeneration of

the cells were most marked. In the healthy peripheral zone a number of mitotic figures were observed in the parnechymal cells. There was moderate proliferation of the Kupffer cells of the sinusoids. Frequently the sinusoids were collapsed due to pressure from the swollen parenchymal cells.

In the case of the litter No. 20 whose parent was deprived of the supplement of yeast in the diet after the 10th day the liver showed extensive necrobiosis of the parenchyma involving the central and the midzonal regions. The cytoplasm of these cells took a homogenous pink stain with eosin and many of the nuclei were swollen and stained lightly or were condensed and pyknotic. Some of the nuclei were undergoing karyorrhexis and lysis. Proliferation of Kupffer cells and histiocytes was marked in the necrotic area. Fine globules of fat were frequently seen in the necrotic area. In the healthy peripheral zones of liver cells abutting on the portal tracks numerous mitosis were observed.

The litter (of those animals Nos. 11, 17, 21, 39, 44,) whose mothers were fed on the basic diet with 1 gram of yeast, showed a remarkable lag in the average gain in weights per litter. The young ones took a long time to be weaned completely. No paralysis of the limbs were observed in any of the litter as there was adequate supply of vitamin B_I in the diet.

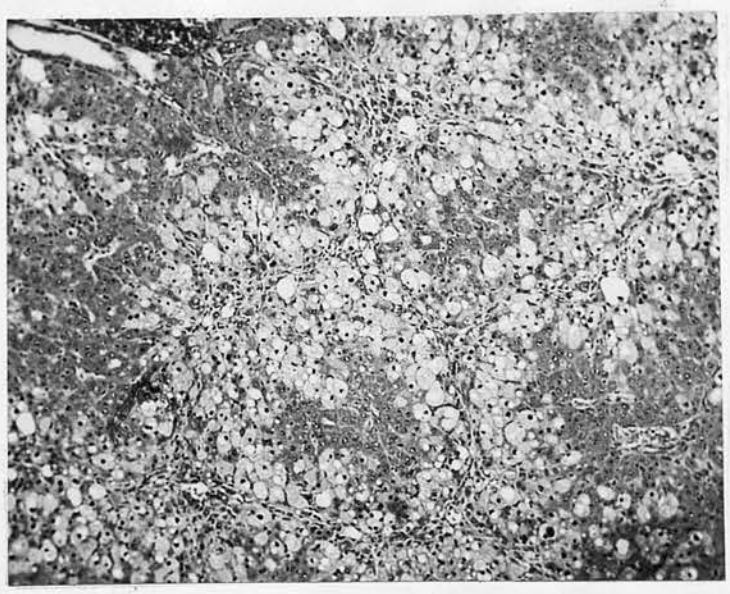


Fig XIII. Liver of Litter 18/VI aged 29 days showing collapse and condensation of reticulum beginning at the centre and extending radially to the neighbouring lobules. There is some collaginization of the reticulum fibres. Except for islands of healthy liver cells at the periphery of the lobule the rest show marked hydropic degeneration. The mother of the animal was fed on special diet and received 0.2 c.c of carbon-tetra-chloride twice weekly.

H and E x 80.

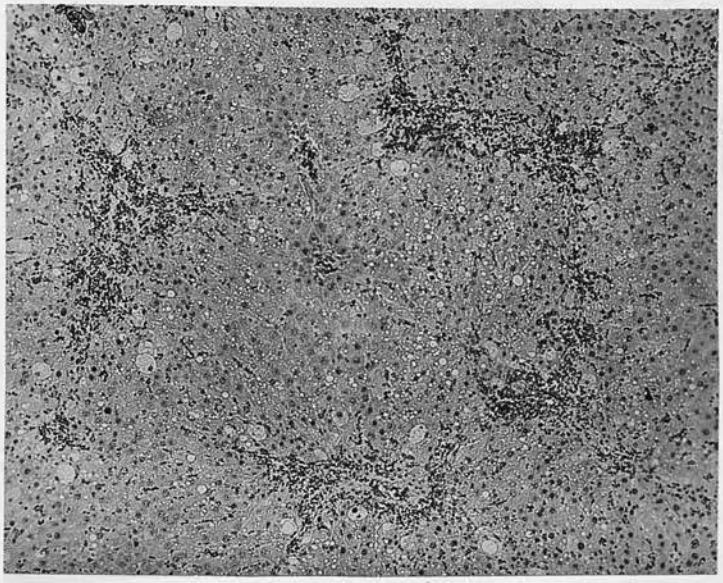


Fig XIV. Liver of rat 18 showing slight necrosis with cellular infiltration at the centre of the lobules. When compared to the liver of its litter the changes here are slight.

H and E x 80

The changes in the liver of these were most marked during the second, third and fourth week of their life, and varied from moderate hydropic degeneration to extensive necrotic changes of the parenchyma. In those animals where the hydropic degeneration was extensive it was always associated with some degree of fatychange and necrosis usually of a few cells in the central zone. The cells either appeared foamy or as unstained empty vacuoles with centrally or peripherally placed nuclei depending on the degree of the degeneration. The neuclei were opaque, and deeply basophilic, or were shrunken and pyknotic. The peripheral zone of cells in most cases were healthy and well stained. In those cases where the necrosis was the more prominent feature three zones were usually made out in the lobule: A central zone of cells undergoing necrotic changes which extended outward probably along the course of the sublobular veins to the adjacent lobules. The cytoplasm of the cells in this area was homogenous and stained pink with eosin. The nuclei were seen to be in various stages of disintegration. Frozen sections stained with Sudan III. showed a fine dispersion of fat globules in this area. The intermediate zone consisted of a layer of single cells which were marked-ly hydropic. The peripheral zone of healthy cells were distributed chiefly around the portal tracts, and were stained a normal bluish pink. In the portal tracts the vein, artery, and duct were unaffected.

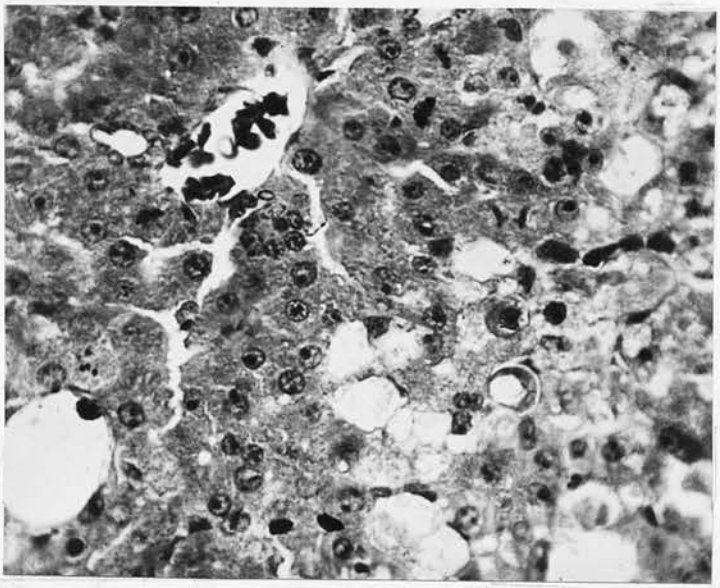


Fig XV. Liver of litter 20/II aged 23 days showing necrosis and hydropic degeneration of liver cells in the centre of the lobule, and healthy cells at the periphery. Numerous mitotic figures are seen in the field.
H and E x 500.

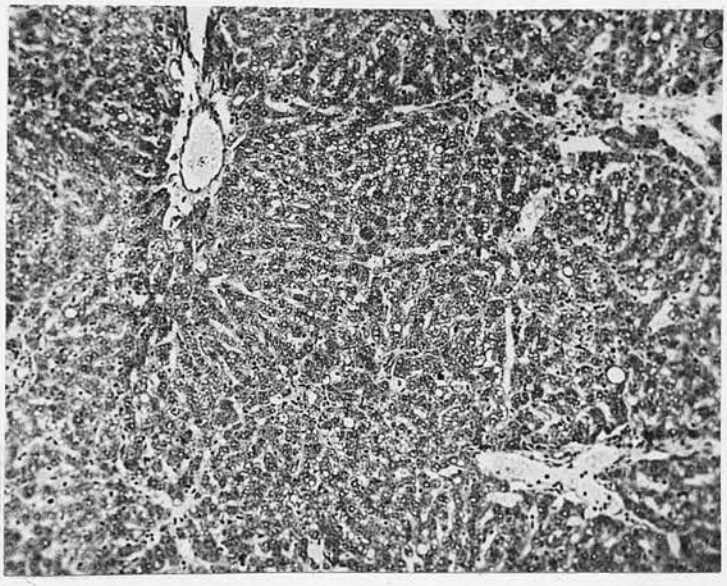


Fig. XVI. Liver of rat 20 which had received 9 injections of 0.2 c.c. of carbon-tetra-chloride at interval of 3 to 4 days during lactation. Liver cells show slight fatty change. No necrosis or hydropic degeneration is observed.

H and E x 80.

The litter of the animal, No. 18, which had 0.5 grams of marmite thrice a week along with the basic diet showed the same retardation of growth as seen in the previous set of litters; but the changes in the liver were slightly different. In the second and third week hydropic degeneration of the cells was the outstanding feature in the liver; but in the third and fourth week the appearance of the section was that of a pre-cirrhotic liver, monolobular in type. There was collapse and condensation of the reticulum of the central zone with proliferation of fibroblasts radiating outwards along the course of the sub-lobular vein to the adjacent lobules. In sections stained with Heidenhain's azan stain a few bands of collagenized fibrous tissue were seen in the fibroblastic extension. The parenchymal cells, except for a narrow zone around the portal canal showed varying degrees of hydropic change. There was a slight cellular increase in the portal tracts.

The litters of the two animals No. 33 and 79 which were fed on basic diet supplemented with 3 grams of meat thrived well and the average gain in weight did not lag far behind the controls in the animal No. 79, whose ration of meat was continued. Whereas in the second animal No. 33, where the meat was discontinued on the 16th day there was a definite retardation subsequently in the weight of the litter.

The histological picture of the liver in the two litters was similar in appearance during the

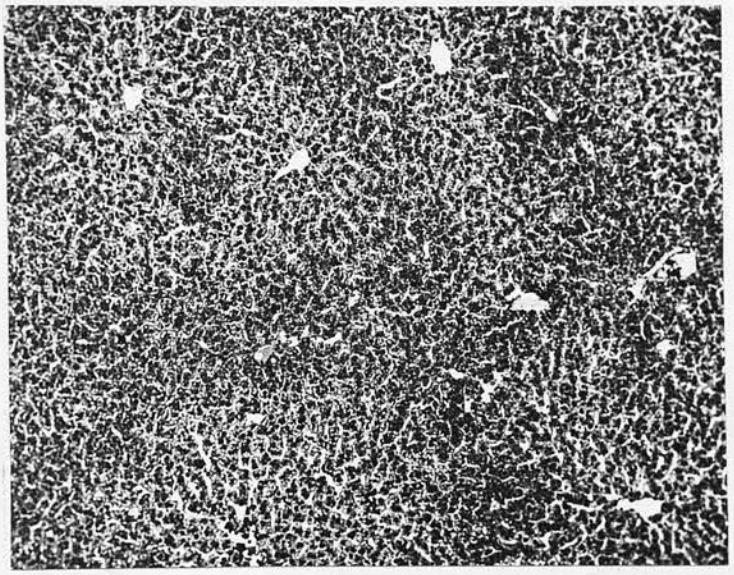


Fig XVII. Liver of litter 33/II aged 16 days whose mother was fed on basic diet and 3 grams of meat while receiving 0.2 c.c. of carbon-tetra-chloride twice weekly at the same time. Slight fatty change at the centre of the lobule is observed. Otherwise the liver cells are well preserved. H and E x 20.

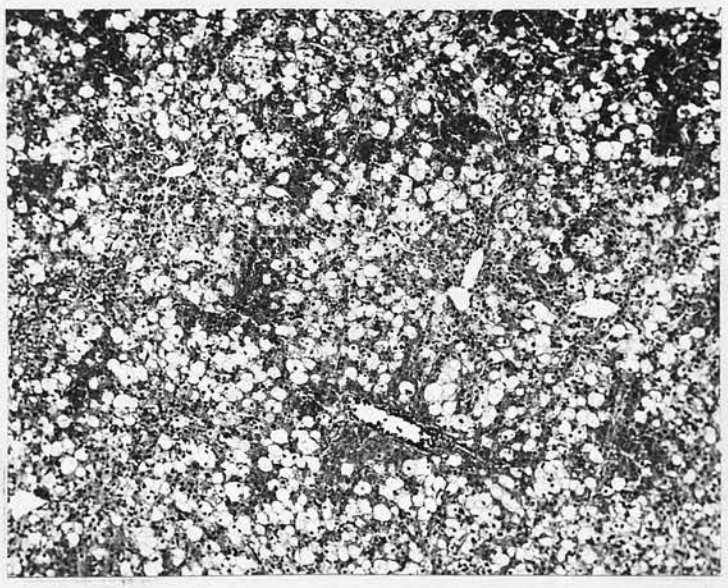


Fig.XVIII. Liver of litter 33/III aged 23 days showing the marked degenerative changes in the liver when the meat in the diet of the mother was discontinued from the 16th.* Three zones can be distinguished in the lobule. The central zone showing necrosis. The middle zone where the cells are hydropic and the peripheral healthy zone of liver cells abutting on the Glisson's Sheath.

H and E x 80.

*16th day of lactation.

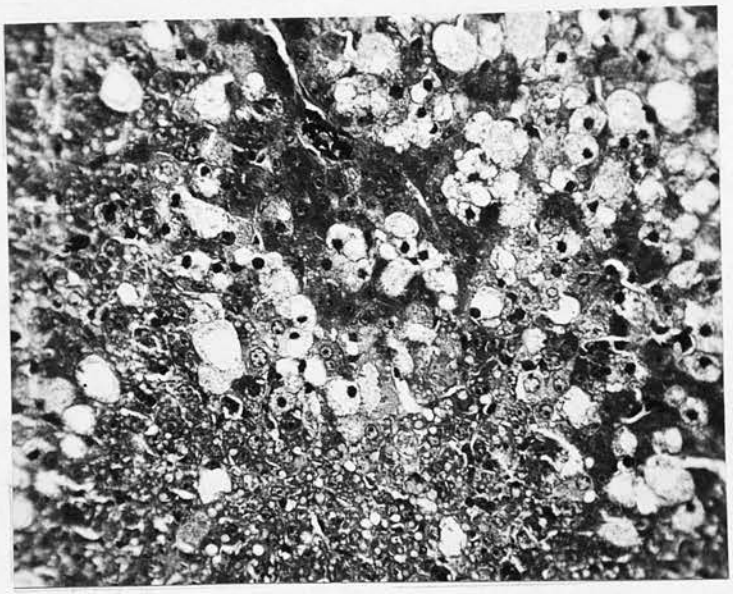


Fig. XIX. Liver of litter 39/II aged 26 days whose mother was on special diet and was receiving injections of Carbon-tetra-chloride. Note the area of central necrosis mid-zonal hydropic degeneration and the peripheral healthy zone.
H and E x 300.

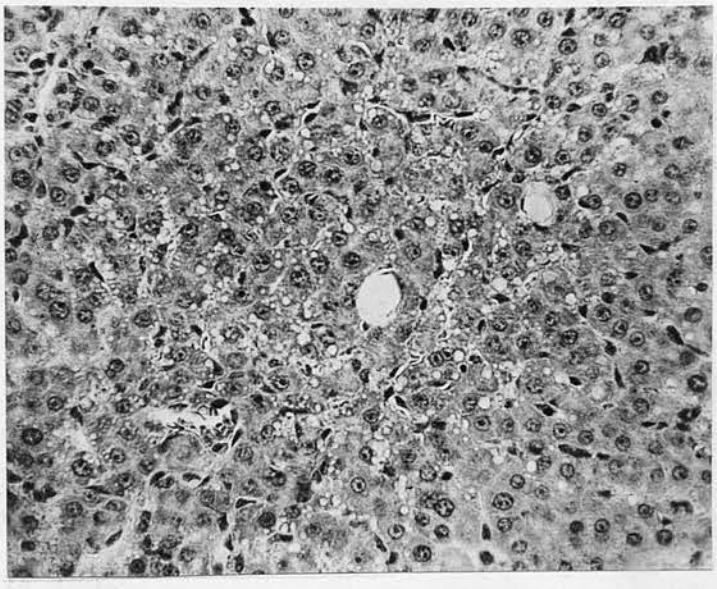


Fig XX. Liver of litter 79/III aged 24 days whose mother was fed on the basic diet supplemented with 3 grams of meat daily while receiving 0,2 c.c. of carbon-tetra-chloride twice weekly at the same time. Parnchymal cells are well stained and healthy. There is only a slight infiltration of fat in the cells.
H and E x 300.

first two weeks. There was slight fatty change in the cells of the central and midzonal regions but the nuclei were well stained and there was no evidence of serious liver damage. The wall of the radicles of the hepatic vein was relaxed. The portal veins were distended with red cells, but the sinusoids were collapsed and empty. There was no apparent proliferation of Kupffer cells. In the third and fourth weeks the litter of the animal No. 79, which had meat in its diet, continued to show the same changes in the liver while the litter of the animal No. 33, which was deprived of the supplementary meat after the 16th day showed extensive hydropic and fatty degeneration of the parenchymal cells of the liver involving the whole lobule leaving only islands of unaffected liver cells at the periphery around the portal tracts. There was some collapse of liver cells and condensation of the reticulum into irregularly radiating bands which spread out from the centre; but there was no evidence of collagenization of the reticulum fibres. The cells near the portal tracts showed mitotic figures, and a few were binucleated.

The litter of the rat No. 83 which was fed with 3 grams of casein in addition to the basic diet showed a moderate gain in weight each week. The liver of the young during the first week was markedly hydropic in the central and midzonal regions of the lobule which over shadowed the fatty change that was present in some of the cells. Numerous foci of

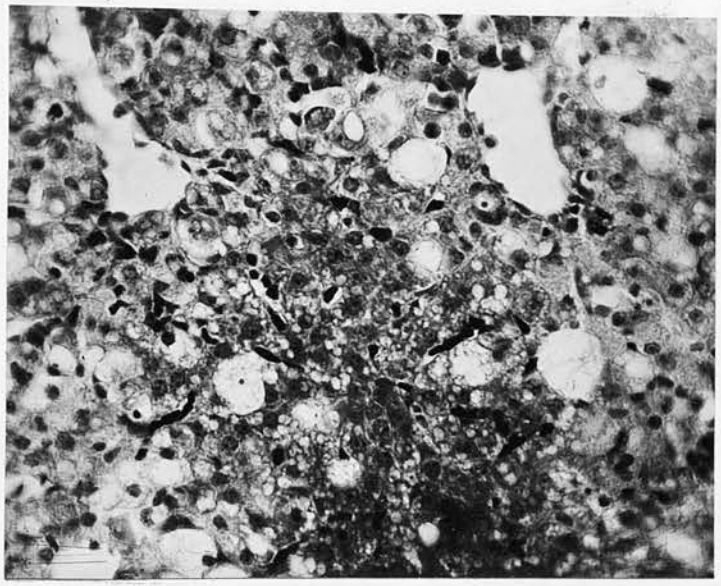


Fig XXI. Liver of litter 83/II aged 6 days whose mother was fed on the basic diet supplemented with 3 grams of casein during the experiment. The cells show marked hydropic degeneration and fatty change in the central and mid-zonal regions.

H and E x 350.

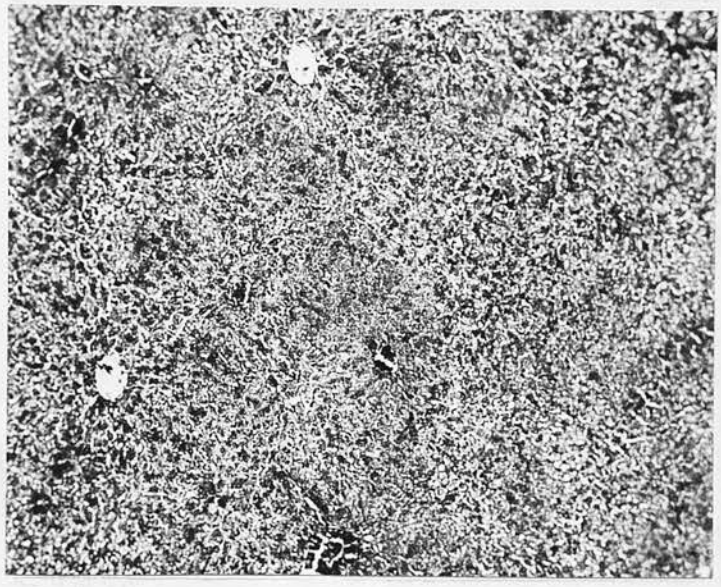


Fig XXII. Liver of litter 83/VI when it was 22 days old. The degenerative changes are less intense. Moderate but diffuse fatty change is seen in all the liver cells. There is no hydropic degeneration or necrosis.

H and E x 75.

haemopoesis were distributed throughout the substance of the liver but these were not affected. The parenchymal cells at the periphery were free from these changes; In the third and fourth weeks the liver damage was much less in evidence. There was no hydropic degeneration but numerous fine globules of fat were seen to be widely dispersed in the cells throughout the lobule. There was no necrosis, or signs of regeneration of liver cells.

Effect on the parent. There was a gradual decline in the body weight of all the experimental animals; but in no case did the injections cause the death of the animal. The liver damage varied slightly in the individual cases. Microscopically the change frequently seen was moderate fatty degeneration of the parenchymal cells either central or diffuse in distribution. Very rarely a few liver cells in the centre of the lobule had undergone necrosis, with subsequent proliferation of histocytes and fibroblasts limited to the affected region. There was no increase of fibrous tissue in any of the livers. The portal tracts showed no cellular infiltration and the cells in the outer and mid-zones of the lobule were healthy.

SUMMARY.

(1) The effect on the liver of sucklings whose mothers were fed on polished rice and vitamins supplemented with either yeast, meat or casein and subjected at the same time to the action of carbon-

tetra-chloride were studied.

(2) Extensive necrosis and hydropic degeneration were observed in the livers of the sucklings whose mothers were partially deprived of either yeast or meat in the diet. The general effect on these animals was to produce a striking retardation of their growth.

(3) Even on the fifth week the young were not completely weaned, and the livers continued to show degenerative changes

(4) The greater the amount of yeast, meat, or casein in the diet of the mother the less the degenerative changes observed in the liver of the suckling; but of the three diets casein had the least protective action.

(5) The livers of the nursing mothers who were receiving the same dose of Carbon-tetra-chloride as the nonpregnant control animals showed only slight evidence of liver change in marked contrast to the extensive necrotic and degenerative changes seen in the controls.

Factors that oppose the development of infantile cirrhosis.

Introduction.

A review of the literature on 'infantile cirrhosis" revealed that a diet of cereals other than polished rice or a diet of polished rice with animal proteins produced a definite immunity against this disease. This hypothesis accords with the observed facts that :-

(1) The disease is not seen in districts where the cultivation is dry, i.e., cultivation of wheat and millets.

(2) The immunity from the disease enjoyed by the poorer class of people whose staple diet is the cheaper millets and parboiled unpolished rice, with the addition now and then of animal protein.

(3) The disease is unknown among the European* and Anglo-indian* Communities in India, and occurs less frequently in Mohammedans and Indian christians who take a mixed diet of animal protein and polished rice.

This hypothesis does not take one far in arriving at a definite conclusion regarding the specific factor or factors that are present in the rice germ or animal protein which inhibits the action of a cirrhogenic toxin on the liver.

*Green Armytage has reported cases in Anglo-indians and Europeans, but since he observes that strict limitation in diet or semi-starvation for a week or 10 days cured the disease it is evident that the condition mentioned by him is not 'infantile cirrhosis'.

Various substances have been credited with having a protective action on the liver. It was reported by Opie and Alfred (1913) that fats increased the susceptibility of rats to chloroform; but on the other hand carbohydrates were found by Graham (1915) to protect the liver against chloroform. Davis and Whipple (1919) found that in starving dogs chloroform produces a more deleterious action on the liver than in dogs fed with either proteins or carbohydrates; but they did not state that glycogen itself has a protective action. Davis (1923-24) suggested that glucose exerted a protective action on the liver; but Chandler and Chopra (1926) in their experiments on dogs could not demonstrate any protective action from the administration of sugar before carbon-tetrachloride. William Von Glahn and Frederick Flinn from their investigation on the action of lead-arsenate on the liver found that the presence of glycogen in the liver did not influence the changes in the parenchyma. Graham (1920) believed that sodium bicarbonate lessened the susceptibility to chloroform poisoning. Minot and Cutler (1928) demonstrated that a high calcium diet in dogs helped them to withstand carbon-tetra-chloried toxaemia; but the extent of liver damage was not reduced. Wokes (1933) did not find that a high calcium diet exerted any protective action in mice against carbon-tetra-chloried.

In recent years the importance of choline in the diet to prevent the fatty infiltration of livers

due to damage by chloroform or carbon-tetra-chloride has been emphasized by Best, MacLean and Ridout (1935), Barrett Best, MacLean and Ridout (1939-40) and others. However they do not state that choline protects against liver damage or that it can prevent necrosis of the liver cells. Forbes, Neale and Scherer (1936) found that large doses of choline did not protect the liver against carbon-tetra-chloride and chloroform.

Of more interest is the claim made by Sato(1926) of a liver harmone "yakriton" prepared from ox liver which prevented necrosis of liver cells. Later Forbes, Neale, and Scherer (1936) prepared a liver extract which reduced the susceptibility of rats to carbon-tetra-chloride and chloroform. Forbes and McConnell (1937) identified the specific substance as sodium-xanthine. Further experiments by Barrett, MacLean and McHenry (1938) confirmed this observation. O. Garth Fitzhugh (1940) found that sodium xanthine exerted a protective action on the liver against carbon-tetra-chloride; but there was no evidence that it stimulated the regeneration of liver cells after damage. Ravdin, Samuel Goldschmidt and Harry M. Vars (1939) from their experiments found that Sodium xanthine was not a specific protective agent, and that the process involved was a stimulation of the protective mechanism secondary to the tissue necrosis at the site of injection. Forbes and Outhouse (1940)observed that the injection of Sodium xanthine,

sodium ricinoleate and tricalcicum phosphate caused a reduction of serum esterase concentration which increased the resistance of the liver to toxins.

Paul Gyorgy and Harry Goldblatt (1939) found that rats fed on a diet lacking only in yeast eludate or yeast extract developed liver necrosis. They observed also that the diet was deficient in purine bases. Arnold Rich and John Hamilton (1940) demonstrated that rabbits fed on a diet which was wanting only in yeast developed cirrhosis. They suggested that the lesion was due to the lack of a specific type of protein present in the yeast. Neale and Winter (1938) observed that besides sodium xanthine, other purine bodies including guanosine, guanine, hypoxanthine and uric acid exerted a protective action on the liver.

In the present study it was decided to administer nucleic acid orally to the mother rats in order to eliminate the additional factor of a local and general reaction which follows subcutaneous injection, and to observe if it will have any protective action on the liver of the sucklings. In addition to this further investigations were carried out in order to observe if nucleic acid administered to non-pregnant adult rats will in any way prevent or lessen necrosis of the liver induced by subcutaneous injections of carbon-tetra-chloride.

Experimental determination of immunity from liver damage in sucklings whose parent is fed on polished rice, vitamins, and neucleic acid and is subjected at the same time to the action of cirrhogenic toxins.

Material and Methods of Study.

Ten albino rats with a total litter of 53 were employed in this experiement. The mated females were segregated in separate cages immediately after the vaginal plug was seen. Nucleic acid was given by stomach tube four times weekly; followed after the second and fourth doses by injections of Carbon-tetra-chloride. Nucleic acid in the form of sodium nucleinate was started on a different day of gestation for each animal, and the dosage varied from 0.2 grams to 0.35 grams dissolved in 2 c.c. of water two to four times a week. The dosage of carbon-tetra-chloride as in the previous experiments was to start the injection with 0.1 c.c. and follow it by 0.15 c.c. twice a week during gestation, and after delivery the dosage was increased to 0.2 c.c. twice a week. The animals were fed on normal diet till they littered; afterwards they were put on the basic diet as in the previous experiments.

When it was decided to administer nucleic acid by mouth the usual procedure of mixing the substance along with the food was not tried as it is almost impossible to state with any accuracy the amount of nucleic acid that the animal might have taken. Furthermore as the

Experimental determination of immunity from liver damage in sucklings whose parent is fed on polished rice, vitamins, and nucleic acid and is subjected at the same time to the action of carbon-tetra-chloride.

Animal No.	Diet		Nucleic Acid in grams		The day of gest when Nuc.Acid & c.t.ch.were started.	Dose of c.t. chlon. in c.c.		No. of Litter	Age of Litter when sacrificed	Wt. of Litter in Gms	Wt.of Liver	Estimated normal wt of Liver	Wt. ratio enlargement.
	Gest	Lact	Gest	Lact		Gest	Lact						
84	Normal	Rice + Vitamins	0.35 gms 4 times a wk.	0.35 gms 4 times a wk.	Nucl Acid on 14th Carb.T. Chl.on 15th day	0.3 c.c total dose	0.2 c.c 2 times a wk.	84/I	6 days	8.2	0.3	.395	.8
								84/II	6 days	8.1	0.3	.39	.7
								84/III	15 days	16.2	0.66	1.07	.6
								84/IV	15 days	15.4	0.68	1.1	.6
								84/V	22 days	22.2	1.3	1.7	.8
								84/VI	22 days	22.8	1.5	1.8	.8
87	Normal	Rice + Vitamins	0.35 gms. 4 times a wk,	0.3 gms 4 times a wk	Nuc.Acid 12th Carb.T. Ch.13th day.	0.4 c.c. total dose	0.2 c.c. 2 times a wk	87/I	12 days	1.48	0.5	1.94	.4
								87/II	20 days	20.2	1.2	1.5	.8
								87/III	25 days	25.3	1.7	1.9	.9
89	Normal	Rice and Vitamins	0.2 gms 4 times a wk.	0.3 gms 4 times a wk.	Nuc.Acid on 10th C.T. Chl. on 11th day	0.4 c.c total dose	0.3 c.c 2 times a wk	89/I	11 days	11.1	0.65	.58	1.1
								89/II	11 days	11.4	0.65	.62	1
								89/III	20 days	20.5	1.15	1.6	.7
								89/IV	20 days	20.3	1.05	1.5	.7
								89/V	28 days	26.1	1.2	2	.6
								89/VI	28 days	27.2	1.25	2	.6

Continued

Animal No.	Diet Gest	Diet Lact	Nucleic Acid in grams Gest	Nucleic Acid in grams Lact	The day of gest when Nuc.Acid & c.t.ch.were started	Dose of c.t.chl in c.c. Gest	Dose of c.t.chl in c.c. Lact	No. of Litter	Age of Litter when sacrificed	Wt.of Litter	Wt.of Liver	Estimated normal wt of Liver	Wt.ratio enlargement
90	Normal	Rice and Vitamins	0.2 gms 4 times a day	0.25 gms 4 times a day	Nuc.Acid on 14th Carb.T. Chl.on 15th day	0.4 c.c total dose	0.2 c.c. 2 times a week	90/I	9 days	9.6	0.32	.46	.7
								90/II	9 days	9.4	0.3	.45	.7
								90/III	20 days	17.6	1.2	1.3	.9
								90/IV	20 days	17.	1.	1.29	.8
								90/V	28 days	24.8	1.65	1.85	.9
								90/VI	28 days	23.4	1.53	1.7	.9
94	Normal	Rice + Vitamins	0.2 gm 4 times a wk.	0.2 gm 4 times a wk.	Nucl.Acid on 8th Carb.T. Chl.on 9th day	0.55 c.c. total dose	0.2 c.c 2 times a wk.	94/I	8 days	14.	0.5	.94	.5
								94/II	8 day	13.5	0.51	.9	.6
								94/III	11 "	15.1	0.55	1.06	.5
								94/IV	11 "	14.8	0.53	1.04	.5
95	Normal	Rice + Vitamins	0.3 gm 4 times a wk.	0.3 gm 4 times a wk.	Nucl.Acid on 6th Carb.T. Ch. on 7th day	0.4 c.c total dose	0.2 c.c. 2 times a wk.	95/I	9 days	12.2	0.37	.74	.5
								95/II	9 "	12.6	0.38	.76	.5
								95/III	15 "	15.8	0.7	1.11	.6
								95/IV	24 "	27.1	1.85	2	.92
								95/V	32 "	34.3	2.2	2.6	.8

Continued

Animal No.	Diet		Nucleic Acid in grams		The day of gest when Nuc.Acid & c.t.ch. were started	Dose of c.t. chl. in c.c.		No. of Litter	Age of Litter when sacrificed	Wt. of Litter	Wt. of Liver	Estimated normal wt of Liver	Wt. ratio enlargement.
	Gest	Lact	Gest	Lact		Gest	Lact						
96	Normal	Rice + Vitamins	0.2 gms 4 times a wk.	0.2 gms 4 times a wk.	Nuc.Acid on 6th Carb.T. chl on 7th day	0.65 c.c. total dose	0.2 c.c 2 times a wk	96/I	7 days	7.6	0.28	.38	.9
								96/II	7 "	7.9	0.28	.39	.7
								96/III	18 "	13	0.6	.86	.7
								96/IV	27 "	20.2	1.45	1.5	.9
								96/V	27 "	20.5	1.5	1.55	1.
99	Normal	Rice + Vitamins	0.2 gms 3 times a wk.	0.2 gm 3 times a wk.	Nuc.Acid on 11th Carb.T. Ch. on 12th Day	0.4 c.c total dose	0.2 c.c. 2 times a wk.	99/I	7	7.8	0.4	.38	1.1
								99/II	7	7.9	0.4	.39	1
								99/III	12	10.8	0.5	.26	1.9
								99/IV	12	11.2	0.6	.6	.1
								99/V	18	11.8	0.45	,66	,7
								99VI	30	21.5	1.25	1.7	.7

Continued

Animal No.	Diet		Nucleic Acid in grams		The day of gest when Nuc.Acid & c.t.ch.were started	Dose of c.t. ch. in c.c.		No. of Litter	Age of Litter when sacrificed	Wt. of Litter	Wt.of Liver	Estimated normal wt of Liver	Wt. ratio enlargement
	Gest	Lact	Gest	Lact		Gest	Lact						
107	Normal	Rice + vitamins	0.35 gms 4 times a wk	0.35 gms 4 times a wk	Nuc.Acid on 12th Carb T. chl.on 13th day	0.4 c.c. total dose	0.2 c.c. 2 times a wk.	107/I	12	11.2	0.35	.64	.5
								107/II	12	11.9	0.4	.66	.6
								107/III	21	11.1	0.92	1.24	.8
								107/IV	30	23.6	1.45	1.5	.9
								107/V	30	23.2	1.4	1.5	.9
108	Normal	Rice + vitamins	0.35 gms 4 times a wk	0.35 gms 4 times a wk	Nuc.Acid on 10th Carb.T. Chl.on 11th day	0.4 c.c total dose	0.2 c.c. 2 times a wk	108/I	0	5.2	0.3	.28	1.1
								108/II	0	5.3	0.3	.28	1.1
								108/III	11	11.2	0.35	.6	.6
								108/IV	11	11.4	0.35	.62	.6
								108/V	20	12.5	0.81	1.29	.7
								108/VI	30	28.1	1.55	2.2	.7
								108/VII	30	23.6	1.5	2	.7

salt is unpalatable there was always the risk of putting the animal off its feed by mixing with it, the substance. The present technique was evolved out of many attempts, which were either partially or completely unsuccessful. The following method was found to be very successful since out of more than 300 stomach tube feedings there were only two deaths from asphyxia.

Technique for using a stomach tube on rat without anaesthesia.

Apparatus required:- (1) A two c.c. syringe. (2) A wide bore needle. (3) Capillary rubber tubing which can be obtained from any surgical instrument makers. (4) A piece of string 2 m.m. in thickness and about 9 ins. in length (5) tissue forceps.

Method. The needle is broken off at the stem and the tip of the stem is filed down to form a pointed stalk up which the capillary tubing could be easily pushed, a lubricant of vaseline being used if necessary. About 4 ins. of the rubber tubing will be the correct length. The stem of the needle fitted with the capillary tubing is attached to the syringe in the usual way whenever it is to be used. The piece of string is threaded through capillary tubing an inch long and the tubing is gradually pushed half way up the length of the string. The assistant grasps the rat with the left hand by means of the loose skin over the shoulder and nape of the neck taking care not to throttle the animal through

Fig. XXIII. Technique of employing a stomach tube on the rat without anaesthesia.

excessive pressure on the integument or to immobolize the chest wall. With the right hand he passes the thread over the upper jaw so that the middle of the length of the thread with the capillary tubing forms a loop just proximal to the upper incisors with the result that the upper jaw is held firmly within the loop, and the curved incisors of the upper jaw prevent effectively any attempt on the part of the rat to struggle free. The capillary tubing on the string is to safeguard against injury to the upper lip of the rat. When the stomach tube is about to be passed in, the assistant pulls the upper jaw backward by means of the string and tightens the loop so as to close the nostrils of the animal. The rat always opens its mouth wide to breathe. With the help of the tissue forceps the capillary tubing attached to the syringe is gently pushed down the throat into the stomach. A little pressure on the tongue of the rat with the forceps will prevent any attempt by the rat to eject the tube. At least 2 inches of the length of rubber tubing measuring from the incisors should have passed down before any attempt is made to push in the fluid, otherwise there is always the risk of regurgitation of the fluid and a complication with broncho-pneumonia. If the syringe is held vertically the weight of the piston is enough to inject the fluid gently into the stomach. Caution must be exercised, not to inject too large a quantity of fluid, nor to distend the stomach suddenly by a

quick push of the piston. As a little carelessness in not attending to these details may cause death of the animal from shock. It is best to put in the stomach tube in the morning before the animal is fed, and a maximum quantity of 2 c.c. is well tolerated by rats weighing 150 grams. With a little experience the stomach tube can be passed in and the fluid injected within two to three minutes for a rat.

Observations.

Effect on the litter. In spite of the fact that the mothers were fed on the basic diet the young thrived well, just as well as the litter of the animals which were fed on a normal diet during the experiment. The litter of the animals that received larger doses of nucleic acid showed greater gain in weight than those of the animals which were given a smaller dose. This could only be accounted for by assuming that the nucleic acid plays a great part in the secretion of a milk having growth promoting properties. The young in these experiments were weaned sooner than in the previous ones.

The litter of animal No. 99 which received the minimum dose of nculeic acid, 0.2 grams, 3 times a week, showed during the first and second week wide spread hydropic degeneration of the liver cells throughout the lobule except for a narrow margin of healthy cells abutting on the Glisson's Sheath. The cytoplasm of the affected cells were foamy and had

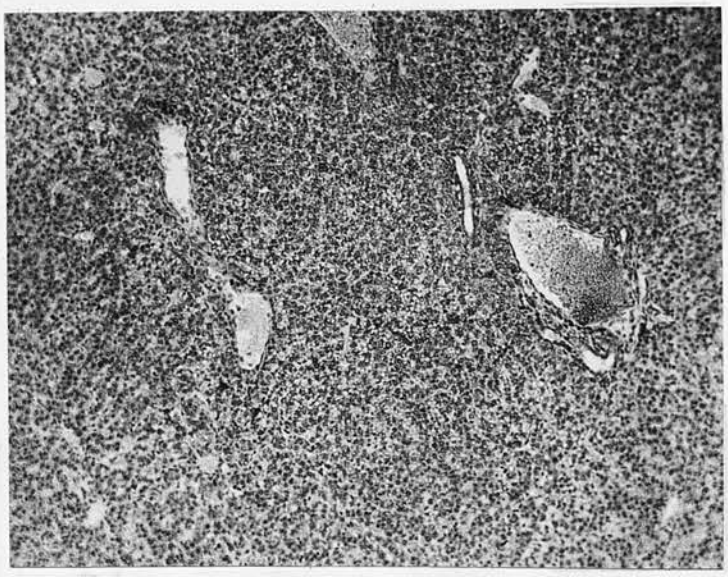


Fig XXIV. Liver of litter 84/IV aged 15 days whose mother was fed on the basic diet and large doses of nucleic acid and was injected with 0.2 c.c. of carbon-tetra-cgloride twice weekly. The liver cells show slight fatty change. No degenerative or necrotic changes are observed.
H and E x 85.

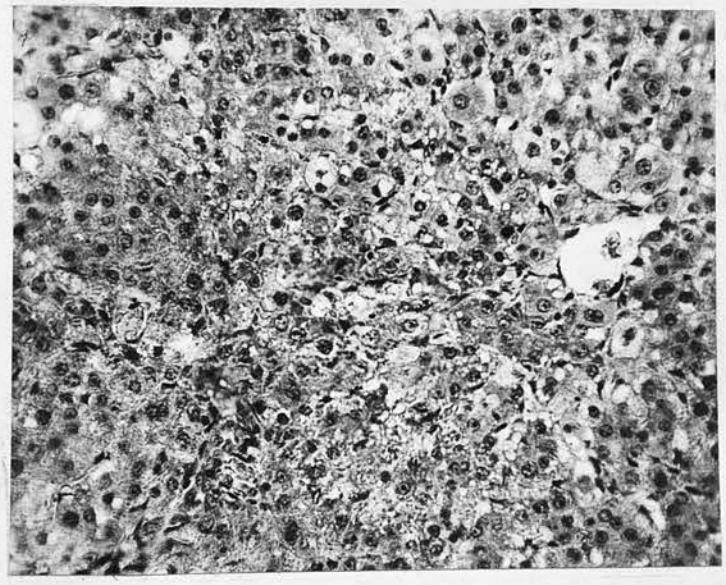


Fig XXV. Liver of litter 84/VI aged 20 days showing slight fatty change in the parenchyma.

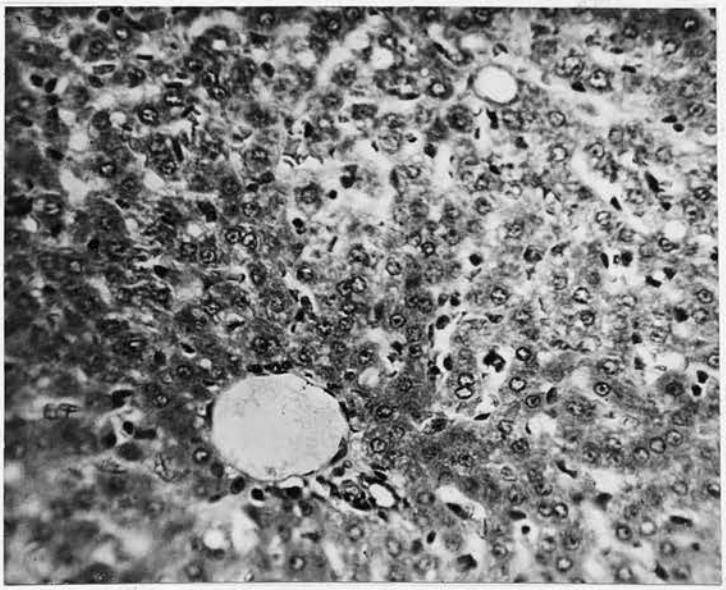


Fig XXVI. Liver of nursing mother rat 87 which was fed on the basic diet and nucliec acid and had received 8 injections of 0.2 c.c. of carbon-tetra-chloride at intervals of 3 or 4 days. A hepatic and a portal venous terminals are in the field. There is no necrosis of the parenchyma, and the fatty change is slight. H and E x 350.

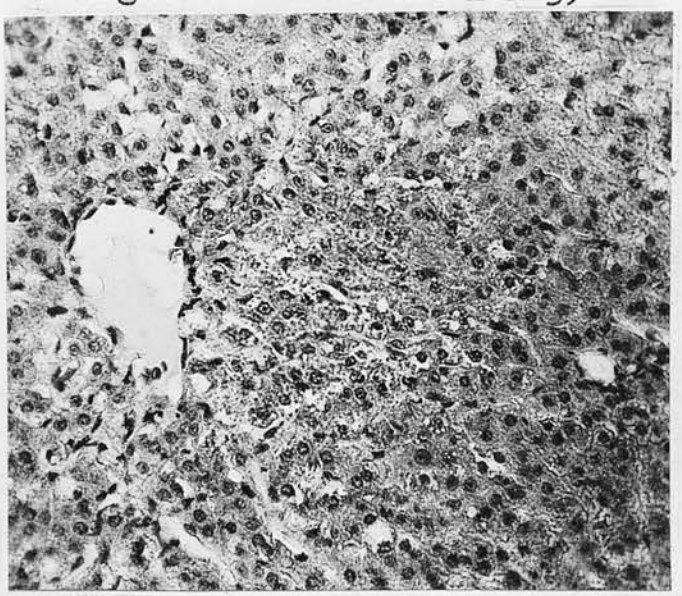


Fig XXVII. Liver of Litter 89/III aged 20 days whose mother was fed on the basic diet and large doses of nucleic acid and received injections of carbon-tetra-chloride. A hepatic and a portal venous terminals are in the field. The cytoplasm of the parenchymal cells is slightly, granular but is well stained. The fatty change is slight; not greater than what is sometimes normally seen in the liver of young animals.
H and E x 300.

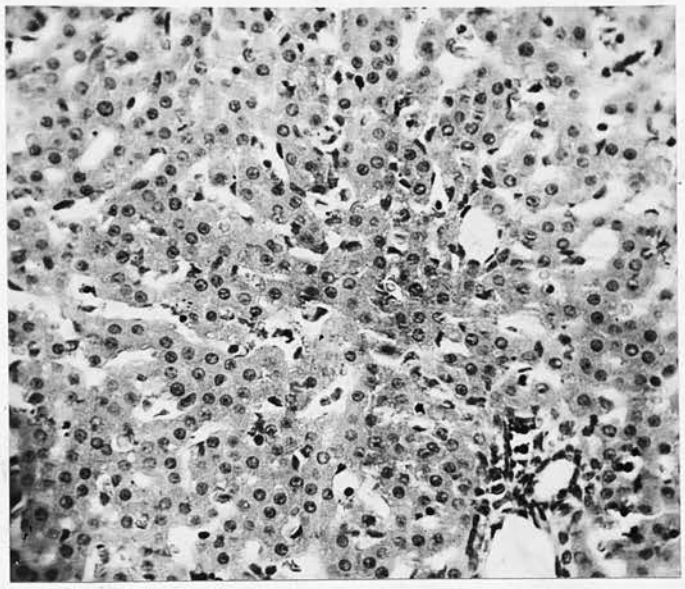


Fig XXVIII. Liver of litter 94/III aged 11 days, whose mother was fed on the basic diet with large doses of nucleic acid and injected with 0.2 c.c. of carbon-tetra-chloride twice weekly. A hepatic and a portal terminals are in the field. The cells are normal.

H and E x 300.

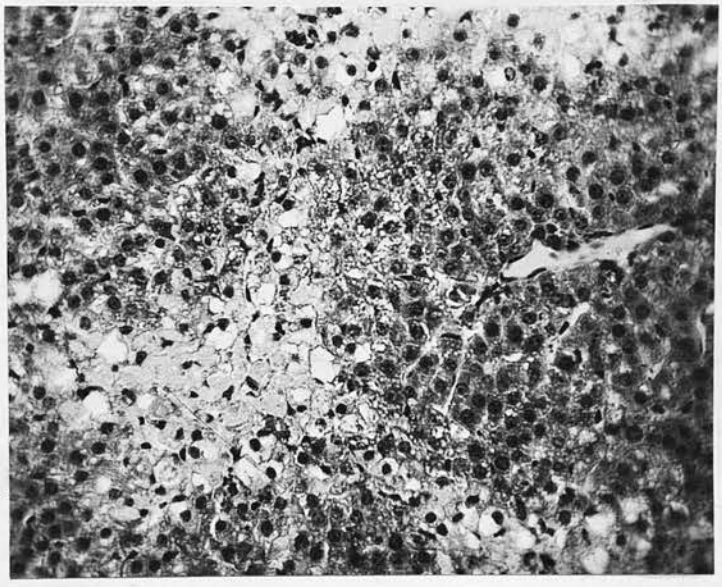


Fig XXIX. Liver of litter 99/V aged 18 days, whose mother was given <u>minimum</u> doses of nucleic acid during the experiment. The cells in the centre of the lobule have undergone coagulation necrosis.

H and E x 300.

centrally placed pyknotic nuclei. The sinusoids were collapsed through pressure by the swollen spheroidal liver cells. The vessels and the duct in the portal canal were healthy. The hepatic venous radicals were relaxed. There was no central necrosis.

During the 3rd week the liver of the same litter showed coagulation necrosis of the centre of the lobule. The affected cells were stained a light salmon pink with eosin. The cytoplasm was homogenous and shrunken with an irregular cell outline. The nuclei were absent in most of the cells and those that were present were seen to be in various stages of disintegration. There was slight proliferation of Kupffer cells and these were polymorphic and swollen. Wide margins of cells around the portal canal were normal. By the 5th week the liver of the young had almost assumed its normal appearance except for a slight increase of the fat content of the cells in the centre of the lobule.

The changes in the livers of the other litters varied but slightly. The litters of these animals receiving the maximum dose of nucleic acid showed the least degenerative changes in the liver.

The liver of the new born was almost normal except for slight vacuolation of some of the parenchymal cells. The cells were slightly basophilic with nuclei containing well stained chromatin and prominent nucleoli. The sinusoids were open and contained some red cells. The haemopoetic foci were healthy and normal in distribution.

During the first and second weeks there was a slight but diffuse fatty change affecting most of the parenchymal cells while a narrow zone of cells around the portal canals remained normal. In some livers the fatty change involved only the centre of the lobules.

In the third and fourth weeks these changes were less noticeable, and were localised to the centre of the lobule. However it was much less in extent than what was seen in the livers of the litters whose mothers were on normal diet during the experiment. The cytoplasm of the cells was slightly granular but the nuclei and the nucleoli were well stained. The branches of the hepatic and portal veins were normal.

By the fifth week no differentiation could be made out between the liver of the control litter and those of the experimental ones. In one, No. 107/V there was marked cellular reaction around the branches of the hepatic vein as well as in the Glisson's Sheath. There was no necrosis or fatty change in the parenchymal cells.

Effect on the parent rat.

There was no instance of abortion or of premature delivery as the dose of carbon-tetra-chloride administered during gestation was less than the toxic dose. During lactation though the animals were receiving 0.2 c.c., a toxic dose, and were being fed on the basic diet containing vitamin B_I; but deficient in B complex, yet the decline in weight was not striking.

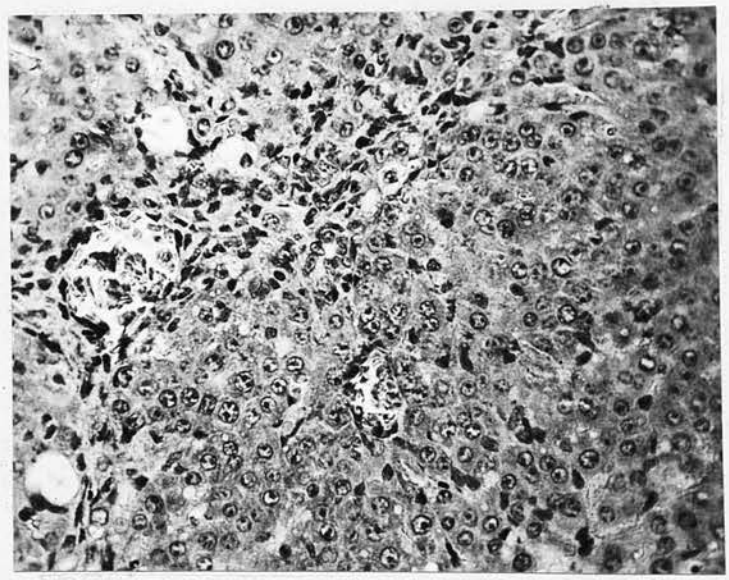


Fig. XXX. Liver of litter 107/V aged 30 days whose mother was given larged doses of nucleic acid during the experiment. The parenchymal cells are healthy; but numerous histocytes are obvious in the portal tract.
H and E x 300.

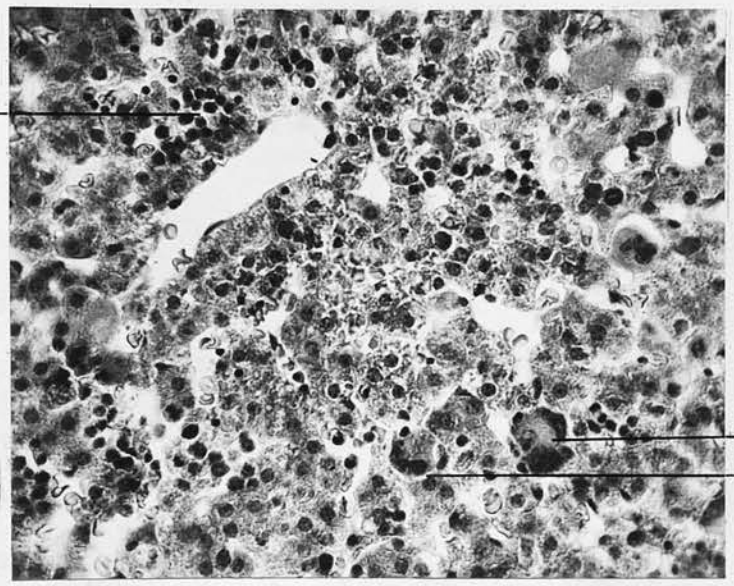


Fig XXXI. Liver of new born litter 108/I whose mother was given large doses of nèuleic acid during the experiment. The cytoplasm of the parenchyma is slightly granular, but well stained and healthy. M. Megakaryocytes. F. Foci of haemopoesis.

The liver damage was chiefly in the nature of fatty change which varied in intensity from slight to extensive. In two animals No. 90 and No. 99 besides the fatty change necrosis of a few cells confined to a narrow fringe of cells around the central veins was observed. The liver of No. 87 was almost normal except for a slight granularity of the cytoplasm of the parenchyma.

SUMMARY.

(1) The effect on the liver of suckling rats whose mothers were fed on polished rice, and vitamins, supplemented with oral administration of definite doses of nucleic acid, and subjected at the same time to the action of carbon-tetra-chloride, were studied.

(2) The observations indicate that the oral administration of nucleic acid to the mother in doses of 0.35 grams four times a week ensures a marked protection against liver damage in sucklings when carbon-tetra-chloride is injected into the mother.

(3) However when the dosage of nucleic acid to the mother is reduced to 0.2 grams three times a week it fails to protect the liver of the sucklings against injury.

(4) Sucklings whose mother is fed on a low protein diet of polished rice show a striking gain in weight with the addition of nucleic acid to the diet of the mother.

(5) As observed in the previous experiments injections of toxic doses of carbon-tetra-chloride to the nursing mother rat does not produce as deleterious an effect on the liver as in the case of the normal adult rats.

Experimental study on the effect on the liver due to oral administration of nucleic acid to non-pregnant adult rats in carbon-tetra-chloride toxaemia.

Material and Methods of Study.

In this experiment 28 albino rats were employed of which 14 were controls. Fourteen experiments were carried out on the whole. Each experiment included the observation of the result of carbon-tetra-chloride toxaemia on a control rat of more or less the same weight and kept under identical conditions in the same cage. In the experimental animals nucleic acid in the form of sodium nucleinate dissolved in distilled water was administered by means of a stomach tube in a single dose, or on consecutive days depending on the dosage of the nucleic acid. A maximum of 0.5 grams in 2 c.c. of distilled water can be given at a time; but larger doses had to be given on successive days. Forty-eight hours after the last dose carbon-tetra-chloride was injected subcutaneously in doses varying from 0.5 c.c. to 0.1 c.c. per 100 grams body weight. The control animals were injected with the same dose of carbon-tetra-chloride at the same time. The animals tolerated these single large doses of carbon-tetra-chloride well; while a much smaller dose at frequent intervals killed the animals. It is also probable that the ability to withstand these large doses, as compared with that of Cameron and Karuneratne (1936) was due to the diet of dog biscuits

Protective action in the growing rats due to the oral administration of Nucleic acid against Carbon-tetra-chloride toxaemia.

Exp. No.	E=Experiment. C=Control	Animal No.	Nucleic Acid in grams	Carb. T.Chl. in c.c.	Body wt. Gms.	Weight of Liver	Estimated normal weight	Weight ratio to normal	% of necrotic area.
1	C_1	B_I	--	0.15	75.5	5.5	4.9	1.1	41
	E_1	A_I	0.5	0.15	75.2	5.2	4.9	1.	34
2	C_2	B_{II}	--	0.15	78.6	5.5	5.05	1.	24
	E_2	A_{II}	0.5	0.15	80.1	6.1	5.1	1.2	20
3	C_3	B_{III}	--	0.1	100.5	7.1	6	1.2	
	E_3	A_{III}	0.4	0.1	83.5	4.3	5.25	0.8	
4	C_4	B_{IV}	--	0.1	100	8.2	6	1.4	
	E_4	A_{IV}	0.4	0.1	80.2	4.1	5.1	0.8	
5	C_5	B_V	--	0.1	125	6.0	7.1	0.8	
	E_5	A_V	0.6	0.1	120	5.3	6.9	0.8	
6	C_6	B_{VI}	--	0.1	112	5.4	6.6	0.8	32
	E_6	A_{VI}	0.6	0.1	110	5.	6.5	0.7	18

Protective action in adult rats due to the oral administration of nucleic acid against carbon-tetra-chlorid toxaemia.

Exp. No.	C=Control E=Experiment	Animal No.	N. Acid in grams	Carb.T. Chl.in c.c. per 100 gm. body wt.	Body wt. in Gms	Weight of Liver	Estimated normal wt. of liver	Wt. ratio to normal	% of Necrotic area.
1	C_7	76	--	0.5	240	15.5	11.45	1.4	33
	E_7	77	1.0	0.5	220	12.7	10.35	1.2	13
2	C_8	82	--	0.25	245	15.1	11.5	1.3	51
	E_8	81	1.2	0.25	300	20.1	13	1.5	25
3	C_9	92	--	0.25	146	7.1	8	0.9	34
	E_9	91	0.3	0.25	143	8	7.8	1	14
4	C_{10}	101	--	0.2	201.5	11.4	10	1.1	
	E_{10}	103	1.0	0.2	203	9.5	10	0.95	
5	C_{11}	104	--	0.2	149.5	8.4	8.1	1	
	E_{11}	102	1.2	0.2	150	7.1	8.1	0.9	
6	C_{12}	106	--	0.2	152	8.	8.2	1.	
	E_{12}	105	0.9	0.2	148	7.9	8.1	1.	
7	C_{13}	110	--	0.2	147	7.6	8.05	0.95	
	E_{13}	111	0.5	0.2	148	6.4	8.1	0.8	
8	C_{14}	112	--	0.2	149	7.3	8.1	0.9	
	E_{14}	113	1.8	0.2	148	6.2	8.1	0.8	

which contains a large percentage of horse flesh. The animals were killed 48 hours after the injection of carbon-tetra-chloride and the livers were fixed in 10% formol-saline. Sections from paraffin blocks were stained with Mayer's haemalum and eosin for routine study. Frozen sections were stained with Sudan III. and counter-stained with haematoxylin for fat. In the sections the proportion of necrotic areas to healthy areas were calculated in all the experiments with acute toxaemia. The image of the sections was projected on graph paper, and the nacrotic areas were marked out in pencil. By counting the number of squares in the necrotic and healthy areas the proportion of each was ascertained. For each section a minimum of five fields were studied.

Result.

Toxic doses of 0.25 to 0.5 c.c. of carbon-tetra-chloride produced similar naked-eye changes in the control as well as in the experimental animals. The livers were markedly enlarged inelastic and soft; but they were more so in the case of control animals. The surface was uneven, and pale yellow in colour with red mottling.

Microscopically the liver of the control animals exhibited central necrosis of varying degree characterized by a homogenous or vacuolated cytoplasm which took a pink stain with eosin and karyorrhexis and lysis of many of the nuclei. The extent of necrosis varied in each liver and such areas were fringed by a single

layer of cells showing marked hydropic degeneration. The Kupffer cells were swollen and prominent with pyknotic nuclei and faintly stained cytoplasm. Tne sinusoids were in many cases distended with red cells and the central veins were seen to have ruptured in a few livers. The peripheral zone of cells were well preserved especially those which were nearest to the portal canal. The portal vein, hepatic artery and bile duct were healthy.

The liver of the experimental animals which received the same dose of carbon-tetra-chloride showed a slightly different picture under the microscope. Compared to the controls the extent of necrosis was much less and in a few it was localized to a narrow margin of cells around the central vein. The characteristic feature in many of the livers was the striking increase ofmononuclear cells in the centre of the lobule. It was difficult to state with any certainty if these were proliferated Kupffer cells or histiocytes of other origin. No polymorphs as observed by Bollman and Mann (1931) in their experiments on dogs were noted in these cases. Sometimes a few lymphocytes were seen in the cellular infiltration. Congestion of the vessels was not a marked feature in these livers. A large percentage of the cells were well preserved and took the normal bluish pink stain. No abnormal increase in the mitosis of liver cells was observed. The Glisson's Sheath frequently showed some infiltration with mononuclear cells.

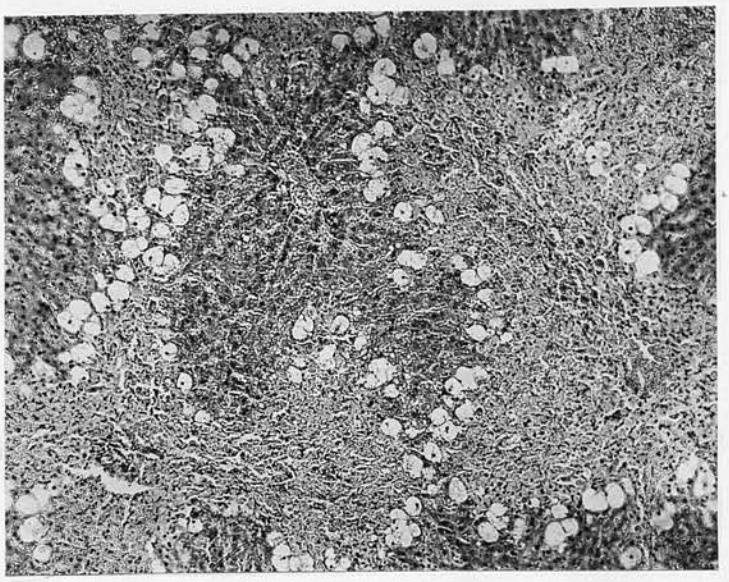


Fig. XXXII. Liver of control rat 76 which had received an injection of 0.5 c.c. of carbon-tetra-chloride per 100 grams body weight. Liver shows extensive necrosis affecting 33% of the lobule. A single layer of hydropic cells separates the normal from the necrotic cells. Very few inflammatory cells are seen in the necrotic area. H and E x 70.

Fig XXXIII. Liver of rat 77 which had been administered orally 1 gram of nucleic acid in two doses 48 hours prior to an injection of 0.5 c.c. of carbon-tetra-chloride per 100 grams body weight. Approximately 13% of the lobule is necrotic. The necrotic debris is infiltrated with numerous histocytes.

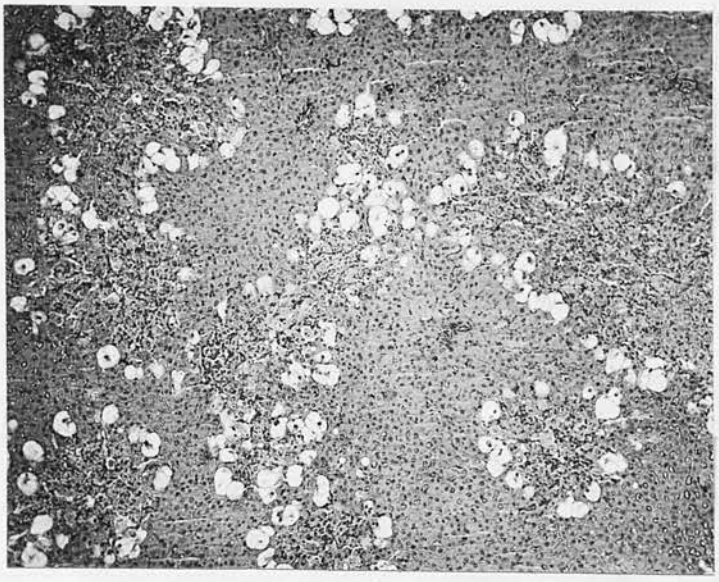


Fig. XXXIV. Liver of control rat 82 which was injected with 0.25 c.c. of carbon-tetra-chloride per 100 grams body weight 51.1% of the lobule is necrotic. There is no infiltration with inflammatory cells.

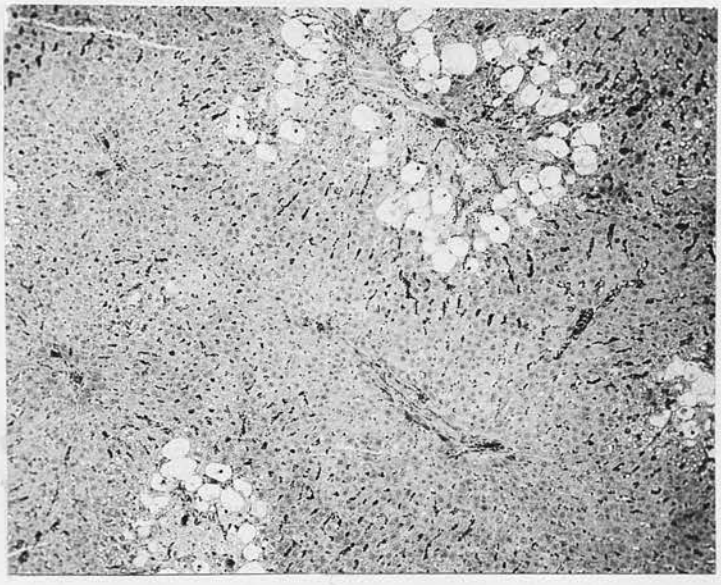


Fig. XXXV. Liver of rat 81 which had been administered 1.2 grams of nucleic acid in two doses orally 48 hours prior to the injection of 0.25 c.c. of carbon-tetra-chloride per 100 grams body weight. 25% of the lobule is necrotic. The necrotic area is infiltrated with a few histocytes.

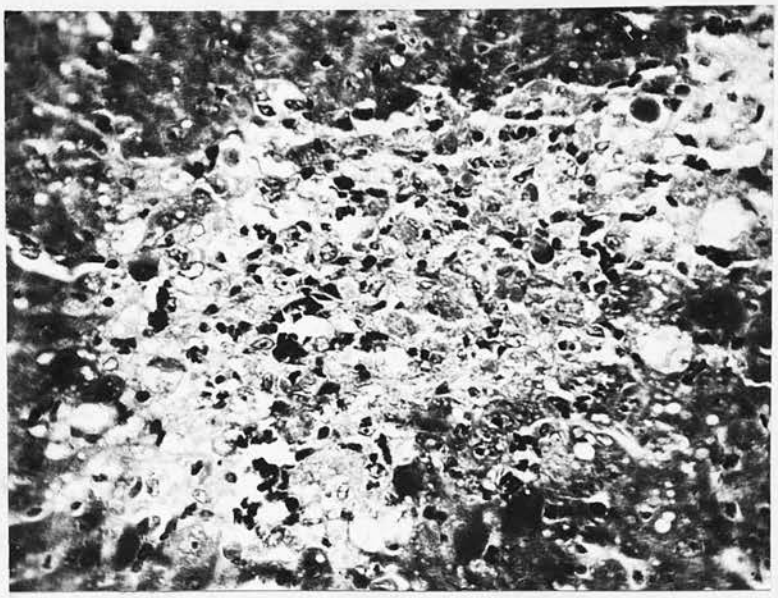


Fig. XXXVI. Liver of control rat 106 which had been injected with 0.2 c.c. of carbon-tetra-chloride per 100 grams body weight. There is marked central necrosis without any apparent cell infiltration.

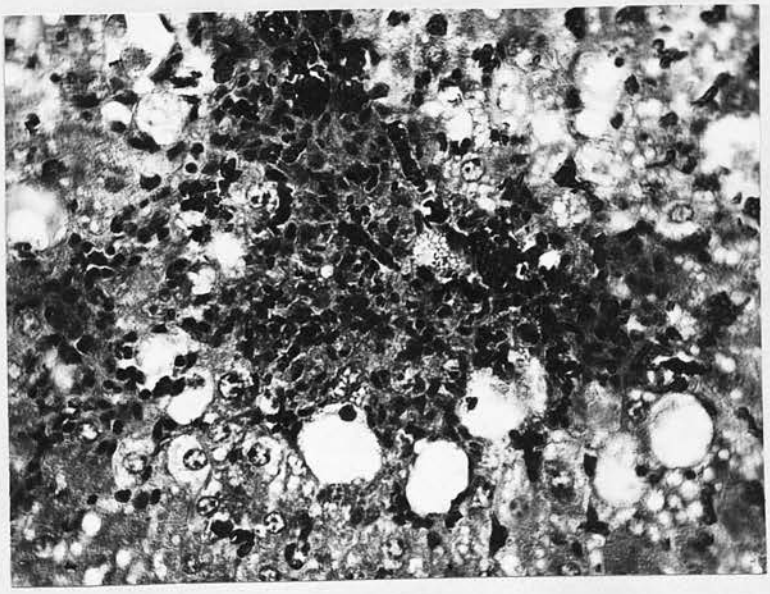


Fig. XXXVII. Liver of rat 105 which had received 0.9 grams of nucleic acid in two doses prior to the injection of 0.2 c.c. of carbon-tetra-chloride per 100 grams body weight. There is a dense infiltration of histiocytes around the hepatic as well as the portal terminals.

In those experiments where the controls as well as the experimental animals received carbon-tetra-chloride in doses of 0.2 c.c. or less, per 100 grams body weight no enlargement of the livers were observed when compared with Hatai's chart 3 for estimated normal weight of the liver. It is possible that the nature of the diet plays a part in the fluctuation of the weight of normal liver. However when compared with the experimental animals, the livers of the control animals always showed greater weight.

Microscopically the control livers showed varying degrees of necrosis, hydropic degeneration and fatty change in the parenchyma. The necrosis was usually confined to a small area in the centre of the lobule; the rest of the central and part of the middle zone were frequently the seat of extensive fatty change. In some, marked congestion and distension of the central veins and the sinusoids were the chief features.

In the experimental animals degenerative and necrotic changes were seen less frequently. Moderate fatty change was observed in many livers; and a few did not show any abnormal change in spite of the large dose of carbon-tetra-chloride. Cellular infiltration with mononuclears was frequently observed, especially around the central veins, and sometimes in the Glisson's Sheath as well. Mitotic figures were not more frequently seen than in normal livers.

SUMMARY.

Oral administration of nucleic acid to experimental rats 48 hours prior to the injection of large doses of carbon-tetra-chloride had the effect of reducing the extent of liver damage as compared with that seen in control animals.

Infiltration of the centre of the lobule and the portal canals with mononuclears was an outstanding feature in the livers of many of the experimental animals.

DISCUSSION.

Liver Damage in Sucklings.

The susceptibility of young animals to chloroform and carbon-tetra-chloride toxaemia has been studied by a few investigators; but there is a marked discrepancy between their evidences. Whipple and Sperry (1909) observed that though animals varied widely in their susceptibility to chloroform the young were more susceptible to this drug. Gardner et al (1925) found that in puppies oral administration of carbon-tetra-chloride was much more toxic than in adult dogs. Whipple (1912) observed that puppies were less susceptible to chloroform anaesthesia in the first three weeks of life. Cameron and Karunaratne (1936) found from their investigations that young rats were able to tolerate bulk for bulk about 15 times the toxic dose for a fully grown animal when administered subcutaneously. A similar observation was made in this laboratory when a few test litters were given subcutaneously injections of carbon-tetra-chloride. Yet the administration of 0.2 c.c. of carbon-tetra-chloride repeatedly to the mother rat produced degenerative changes in the liver of the suckling. The only explanation that can be offered is that given by Cameron and Karunaratne that circulatory relationship may be different in the young animals and adults. This explains the increased susceptibility of puppies to chloroform when given by the mouth; an observation from which it

can be inferred that portal circulation plays a greater part in the nutrition and maintenance of liver cells in the young than the hepatic artery. Apparently either a subcutaneous injection or a toxin inhaled through the lung will affect the liver of the young to a much lesser extent than when it is given by mouth. This hypothesis is borne out by the experimental work of Cameron and Mayes (1930) on very young rabbits in whom ligation of hepatic artery did not result in pale infarct of the liver as it did in the adult. According to Wells (1925) Carbon-tetra-chloride when administered orally occurs in very high concentration in the portal vein and in the liver. Robbins (1929) has made a similar statement. Schultz, Hall, and Baker (1923) found that when chloroform was injected directly into the portal vein of dogs, caused an extensive necrosis where groups of lobules were destroyed, and that the hepatic cells in the periphery of the lobule were chiefly affected. This finding indicates the greater toxicity of the drug when it reaches the liver through the portal stream. It is possible that in the young animals cirrhogenic toxin when injected subcutaneously is detoxicated in other tissues before it reaches the liver through the hepatic artery and portal vein, whereas when it reaches the alimentary tract along with the milk, it is present in the portal circulation in sufficiently high concentrations to injure the parenchyma of the liver. In the present study it was found that as a rule there was more degenerative changes in

the liver of the suckling than what was found in the nursing mother rat which actually received the toxic doses of carbon-tetra-chloride. It is evident that carbon-tetra-chloride was being rapidly excreted through the milk and that only a small percentage of the drug came in contact with the liver cells of the mother. Another observation of interest which was made during these investigations was that trypan blue in a colloidal solution in water was neither excreted through the kidney, nor did it pass through the placenta but on the other hand it was found to pass freely through the mammary secretion. It is not surprising that carbon-tetra-chloride a fat soluble substance should be found in large concentration in the milk of the nursing mother when the drug was administered to it. The result of these experiments justify the suggestion that the probable cause of infantile cirrhosis, taking into account the familial tendency, and the improvement in the condition of the child on cessation of breast feeding, is that it is due to a toxin of maternal origin which injures the liver of the foetus in utero and is continued to be given through the milk in postnatal life.

Polished rice as an etiological factor in liver damage.

From a study of the distribution of infantile cirrhosis one cannot help being struck by the fact that it occurs chiefly in the rice growing district and among the rice eating population. This fact even though it

may have attracted the attention of a few observers, failed to impress the majority, as it was taken for granted that a high carbohydrate diet offered the best protection against liver damage, though the available experimental data does not justify that conclusion. The importance of protein in the prevention of liver damage has not been fully studied up till now, and the observation of Opic and Alford, and others who tried diets of oatmeal, meat and fat in chloroform poisoning are inconclusive as they failed to take into consideration the large percentage of fat in the meat. Meat (mutton leg) contains 15.4% protein, 14.5% fat, 0.8% ash of mineral salts, 51.9% and 17.7% refuse, (McCarrison 1931). They also found that a mixed diet of oatmeal and fat lessened the duration of life of an animal to a greater extent when subjected to chloroform poisoning than a diet of meat. Oatmeal itself contains a fair percentage of proteins, and the germ of the grain has antinecrotic properties. Their third observations that in animals which were subjected to small doses of chloroform those which were fed on meat did not lose weight whereas those which were fed on high carbohydrate or fat diet lost weight, proves the value of meat diet. There is no actual experimental data to prove that a diet of carbohydrate reduces the extent of necrosis in the liver in toxaemias affecting the liver. The experimental production of ascitis by Bollman and Mann in dogs with cirrhosis of the liver by high protein

diet does not indicate increased liver damage, as many causes other than parenchymal damage are involved in the production of ascitis. According to Coudry (quoting Noel 1923) glycogen appears first and in more abundance in the cells adjacent to the central vein in the liver of an animal following the ingestion of carbohydrates; yet it is this region that is first affected in carbon-tetra-chloride, chloroform and many other toxaemias. Cramer and Krause (1913) found that excessive administration of thyroid depleted the liver of its glycogen; yet Cameron and Karunaratne (1935) found no alteration in the susceptibility of albino rats to carbon-tetra-chloride toxaemia after depletion of the glycogen storage of the liver through administration of thyroid extracts. There is no doubt that starvation as well as ingestion of fat increases the susceptibility of the liver to chloroform and carbon-tetra-chloride toxaemia; but it has not been proved experimentally that a carbohydrate diet acts more beneficially than animal proteins devoid of fat. According to McCay (1912) rice is the poorest of cereals in protein content and contains 8% protein, 0.3% fat, 29% of carbohydrate, 0.4% mineral ash, 12.3% water, 0.2% refuse. When cooked it absorbs 3 to 3½ times its weight of water and swells up five times its volume. McCarrison observed "the decrease in the coefficient of protein absorption with diets containing large amounts of rice may be so great that the total nitrogen absorption may fall to little more

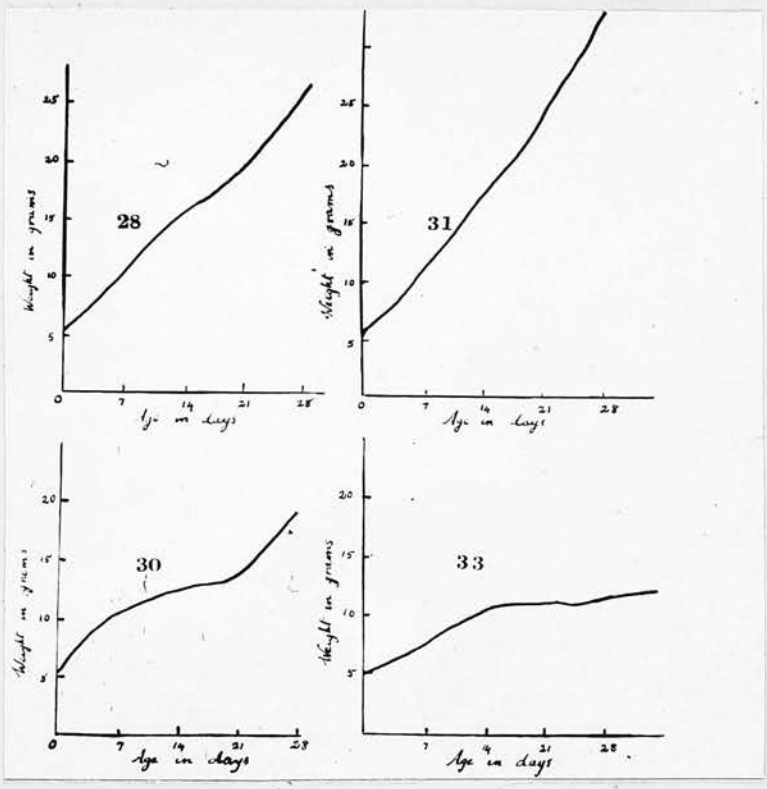


Chart I.

Postnatal growth curves of litters based on the average weight of the surviving young.

28. Growth curve of litter 28 whose parent was on normal diet and was subjected to carbon-tetra-chloride toxaemia.

31. Growth curve of litter 31 whose parent was on normal diet and was subjected to carbon-tetra-chloride toxaemia.

30. Growth curve of litter 30 whose parent was on basic diet with 2.5 grams of yeast during lactation and was subjected to carbon-tetra-chloride toxaemia.
Note the gain in weight of the litter after weaning.

33. Growth curve of litter 33 whose parent was fed on the basic diet and 3 grams of meat for 16 days and was subjected to the action of carbon-tetra-chloride.
Note the absence of gain in weight of the litter after the 16th day.

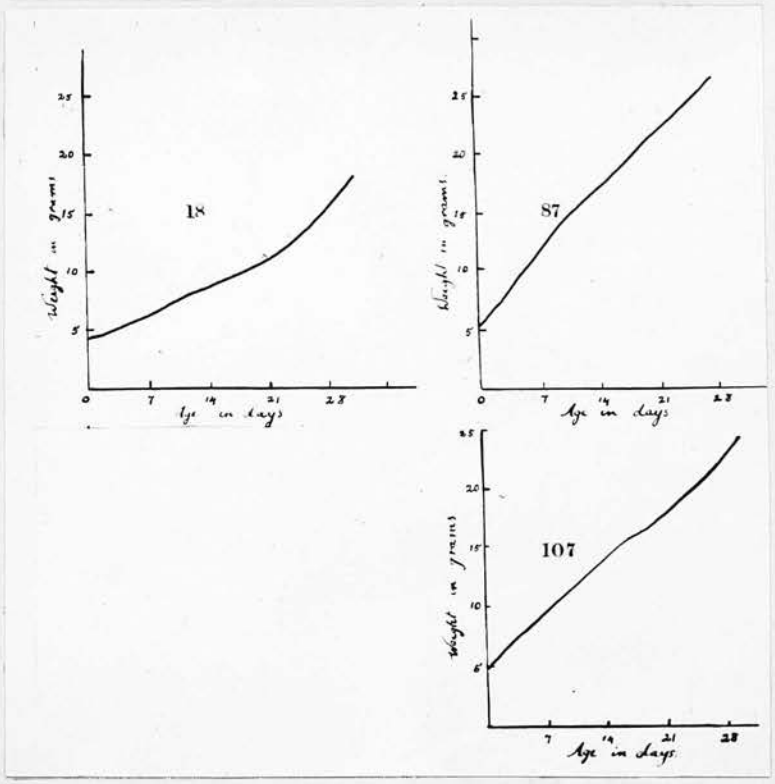


Chart II.

Postnatal growth curve of litter based on the average body weight of the surviving young.

18. Growth curve of litter 18 whose parent was on basic diet + ½ gram of marmite thrice weekly during the experiment. (Carbon-tetra-chloride toxaemia).

87. Growth curve of litter 87 whose parent was on basic diet + 0.3 grams of nucleic acid 4 times weekly during the experiment (carbon-tetra-chloride toxaemia).

107. Growth curve of litter 107 whose parent was on the basic diet + 0.35 grams of nucleic acid 4 times a week during the experiment. (Carbon-tetra-chloride toxaemia).

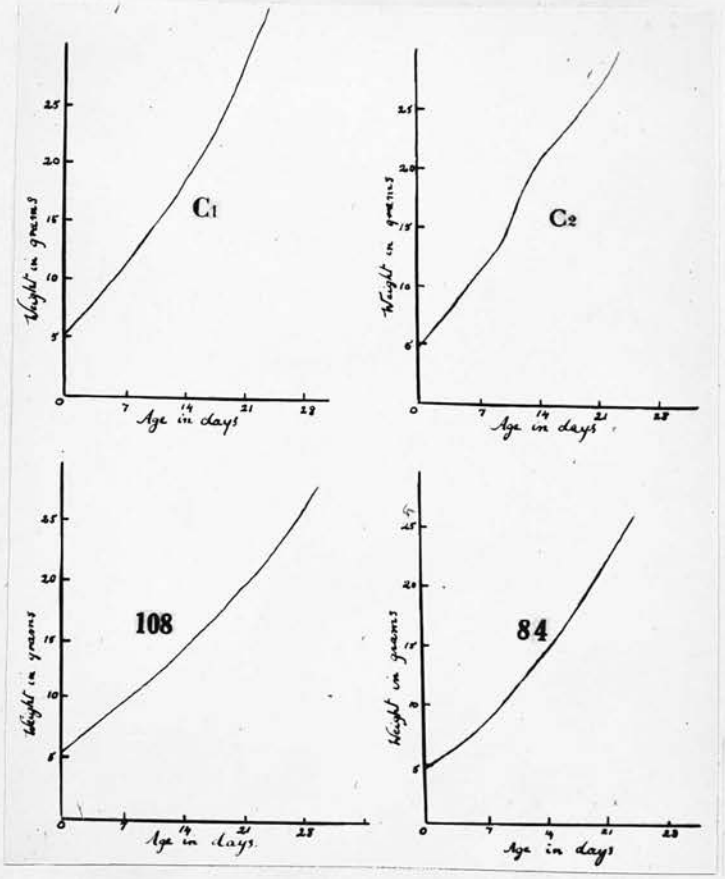


Chart III.

Postnatal growth curve of litter based on the average body weight of the surviving young.

C_1 and C_2 Growth curves of control litters C_1 and C_2 whose mothers were on normal diet and were <u>not</u> subjected to carbon-tetra-chloride toxaemia.

108 Growth curve of litter 108 whose mother was on the basic diet + 0.35 grams of nucleic acid four times weekly during the experiment. (Carbon-tetra-chloride toxaemia).

84. Growth curve of litter 84 whose mother was on the basic diet + 0.35 grams of nucleic acid 4 times weekly during the experiment. (Carbon-tetra-chloride toxaemia.)

than 50% of protein present in the diet. With regard to dhal or pulse which is the main source of protein supply in the Indian rice eaters diet, it was found that an increase of this beyond 5 ounces per day resulted in diarrhoea, since it acted more as an intestinal irritant in larger amounts. The deficiency of protein in the vegetarian rice eaters diet is an important factor in considering the etiology of cirrhosis in tropics especially infantile cirrhosis. Ross A. Gortner (1940) and others found that in cases of selenium intoxication, diet containing 35% casein offered more protection to young white rats than did diet of equal caloric value containing less casein. This observation is of significance in view of the fact that selenium is an element which even in minute doses produces necrosis of liver and later cirrhosis. In the present series of experiments it was found that a diet of carbohydrates in the form of polished rice to the nursing mother rats increased the liver damage in the sucklings.

There are two factors in operation in the genesis of this parenchymal damage.

(1) The cirrhogenic toxin in the form of carbon-tetra-chloride which is being given through the milk.

(2) The absence of a protecting agent in the diet of the mother. The remarkable feature in these experiments was that the absence of the protective factor in the diet of the mother resulted in greater damage to the liver of the suckling than to that of the mother. It can only be inferred that the suckling uses up this anti-necrotic agent more rapidly. It is significant

that the mortality due to infantile cirrhosis should be greatest at the age period of 6 months to 2 years. This high mortality rate due to cirrhosis during that short age period seems to imply that either the affected children are not assimilating enough of the protecting factor in the diet or that it is being rapidly used up in the normal metabolism of the body. The fact that cirrhosis is many times more common among rice eating population than in others favour the view that the supply of this protective factor is deficient in the diet of the infant; but the consideration that this type of cirrhosis is rarely met with after 3 years of age suggests that infants between 6 months and two years use up much more of this protective factor in normal metabolism than adults. It was seen in these experiments that the addition of meat or casein to the basic diet of the mother rat resulted in the reduction of the extent of liver damage in the suckling. Similarly an increase in the percentage of yeast in the diet reduced the liver damage whereas a reduction in the percentage caused marked necrosis of the liver of the suckling. The observation of Paul Gyorgi who found extensive liver necrosis in rats fed on a diet consisting of a large percentage of casein, as well as that of Rich and Hamilton who produced cirrhosis in the liver of rabbits on a diet containing casein in addition to carbohydrates and fast, prove that it is not the protein itself that is the anti-necrotic factor but that it is a substance which can be synthesized

from protein under certain conditions. The conclusion arrived at in the present study was that this substance was present in large amounts in yeast and meat and that it can be synthesized from casein under certain conditions.

The effect of nucleic acid administration in experimental liver damage.

Paul Gyorgy and Harry Gold Blatt (1939) observed that many of the rats which were fed on a synthetic diet containing all the essential vitamins developed necrosis of liver cells, and cirrhosis. They noted that the diet was wanting in yeast as well as in purine substances. The addition of yeast extract prevented such necrosis. A similar observation was made by Rich and Hamilton in the case of rabbits. Von Glahn W.C. and Frederick Flinn (1939) found that yeast reduced the incidence of hepatic cirrhosis in rabbits, produced by ingestion of lead arsenate. However they were not able to state what the protective substance in the yeast may be. Chaikoff, Conner and Biskini (1938) were able to produce fatty infiltration of the liver and cirrhosis in depancreatised dogs kept alive for 2.6 to 5.5 years with insulin injections. On the other hand Kapalam and Chaikoff (1937) found that the addition of pancreas to the diet of these dogs will prevent as well as cure the fatty infiltration of the liver. Channon and Wilkinson (1935) have proved that a low protein intake

produces fatty livers in rats. The question arises whether in the depancreatised dogs it is the interference with the absorption of proteins that is responsible for the liver damage or if there is a specific factor in the pancreas fed to the dogs that prevents the injury. If it is considered that there is a specific substance in the pancreas, the richness of nucleic acid in the pancreas is of special significance. Nakahara Fujiwana and Mori (1939) noted a certain geographical similarity in the occurrance of beriberi and hepatoma in man. Their experiments on albino rats fed on polished rice and subjected to the action of dimethylaminoazobenzol or butter yellow demonstrated that yeast feeding caused some inhibition of liver cancer development, but it was slight when compared to the effect of liver feeding. They also found that large doses of vitamin B_I did not in any way influence beneficially rats fed on polished rice and subjected to the action of butter yellow.

It will be noted that in all the above mentioned experiments there was a complete absence of purine substances in the diets tried, and the articles of diet such as pancreas, meat, yeast and liver which prevented such damage were rich in nucleic acid content. The experiments in the present study were carried out in order to ascertain how far nucleic acid by itself will be able to protect the liver against carbon-tetra-chloride toxaemia. It was

demonstrated in these experiments that lean meat as well as nucleic acid in sufficient amount in the diet of the nursing mother had a marked effect in protecting the liver of the suckling rat against liver damage; but nucleic acid when given in minimum doses was of no value. Nucleic acid was also found to protect the liver of adult rats against damage. This protective action was more marked in the sucklings and in the mature or old rats than in the growing rats.

The question whether or not animals can synthesize the purine derivatives needed for nucleic acid formation has not been so far satisfactorily answered. Robertson from his experiments on albino rats found that the oral administration of thymic nucleic acid and yeast nucleic acid enhanced their longevity, the prolongation being 12½% of normal duration for males and 17% for females with thymic nucleic acid and slightly greater with yeast and nucleic acid. This experiment indicates that at least adult animals are not able to synthesize the optimum amount of nucleic acid. Ackroyd and Hopkins (1916) found that when arginine and histidine were together removed from the diet of rats which had been previously growing on a complete amino-acid mixture there was a rapid loss of body weight. This demonstrates that rats are not able to synthesize the necessary purines from a carbohydrate diet. The production of cirrhosis in rats by Gyorgy and Goldblatt, and in the rabbit by Rich and Hamilton may be due to the fact that rabbits as well

as rats during certain period of their life at least are not able to synthesize nucleic acid from casein to protect their liver against naturally occurring intestinal toxins.

Nucleic acid has been used frequently in the production of leucocytosis experimentally and to some extent therapeutically. Since the isolation by Jackson (1924) of nucleotides in the circulating blood the conception that nucleic acid stimulates the bone marrow in the production of leucocytes has been strengthened Antoncich (1926), Doan (1926), as well as Sabin and Doan (1927-28) held the view that the action is a true stimulation whereas others believe that its action is that of a chemotactic substance exciting the discharge of white cells from the bone marrow. The consensus of opinion seems to favour the view that it is a growth stimulating factor influencing the maturation of the leucocytes. It was found in the present study that administration of yeast nucleic acid to the nursing mother not only had a remarkable influence against liver damage but that it also proved to be an effective substitute for that fraction the lack of which in the polished rice retarded the growth of the sucklings. Funk, Lyli and McCaskey (1911) ascertained by experiments that yeast by itself was not of much value as food; the same remark must apply to nucleic acid also. However it seems to supplement the necessary factors in polished rice both

for protection against liver damage and growth of the sucklings. Furthermore it was found in these experimental studies that in those animals which were given large doses of nucleic acid, mobilization of histiocytes in the affected area of the liver was rapidly carried out, and the cells were also more numerous when compared with the controls. It is possible that the rapid production of mature white cells under the influence of nucleic acid may be a factor in the detoxication of carbon-tetra-chloride, with the result that the injury to the liver is lessened. However it is as yet difficult to specify the exact mechanism of the protection furnished by nucleic acid against liver injury due to toxins.

With regard to infantile cirrhosis the experimental data point to the deficiency of nucleic acid in the diet of the mother as the most important etiological factor. The studies of Paul Gyorgy and Goldblatt as well as those of Rich and Hamilton demonstrate that cirrhosis in animals may be solely due to dietary deficiency. The question whether or not an abnormal toxic factor plays a role in the geneses of 'Infantile Cirrhosis"' or if this second factor is a naturally occurring intestinal toxin, is a moot point. Whatever may be the nature of the toxin it is demonstrated by these experiments that its action on the liver is controlled by the ingestion of substances rich in nucleic acid.

It is suggested that in those cases where successive children of a mother succumb to infantile cirrhosis, an addition of food stuffs rich in nucleic acid to the diet of the mother during pregnancy and lactation will prevent the production of this disease in the offspring.

GENERAL SUMMARY.

1) A review of literature on "infantile cirrhosis" suggests that the disease is due to a toxin in maternal circulation which affects the liver of the foetus and is continued to be given to the child through the milk in post-natal life.

2) It was demonstrated experimentally that it is possible for cirrhogenic toxins to diffuse through the placenta of a pregnant rat, and to cause degenerative changes in the liver of the foetuses.

3) Injections of carbon-tetra-chloride to the nursing mother rats caused injury to the liver of the suckling, and this injury was enhanced when the mother rat was fed on polished rice and the essential vitamins only. The addition of casein, meat and yeast to this diet decreased the extent of liver damage in the sucklings.

4) Nursing mother rats tolerated larger repeated doses of carbon-tetra-chloride than normal adult rats; and the extent of injury to the liver of the mother was slight when compared to that found in the sucklings, and in the control adult rats.

5) The oral administration of large doses of nucleic acid to the mother rat effectively safeguarded the liver of the suckling against injury.

6) This protective action of nucleic acid against carbon-tetra-chloride toxaemia was experimentally demonstrated in the case of adult rats also.

7) The experimental evidence suggests that the etiological factor in the production of 'infantile cirrhosis' is the lack of nucleic acid in the diet of the pregnant and nursing mother.

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P A R T II.

A comparative histopathological study of 'Infantile Cirrhosis' of India with cirrhosis of the liver in infants of Edinburgh.

A comparative histopathological study of "Infantile Biliary Cirrhosis" of India with cirrhosis of the liver in infants of Edinburgh.

INTRODUCTION:-

Infantile Biliary Cirrhosis of India was described for the first time by Sen in 1887, and was examined morphologically by Gibbons in 1888. Gibbons described it as "a form"of biliary cirrhosis as he found it to be different from the biliary cirrhosis known in Europe. He believed that the liver cells had been subjected to the action of some toxin which induced irritation leading to their degeneration, and to stimulation of the connective tissue element. Prof. Poltuf and Prof. Kundart to whom he showed the sections concluded that the disease was a hitherto unknown form of biliary cirrhosis. Castillani and Chalmers (1919) suggested reinvestigation to decide if it was a variety of Kala-agar. Manson & Bahr(1927) believed in the possibility of the cirrhosis being due to infantile Kala-agar. Pearse (1919) and Chandra Lahiei (1936) were of opinion that it was due to B. coli, streptococci or other micro-organism. Tirumurthi and Radhakrishna Rao studied the pathological changes in the liver in this disease in 1934 and in 1933 Ramachandra Rao investigated the histopathology of two cases of Infantile Biliary Cirrhosis, and decided that one was a case of'subacute toxic cirrhosis' and the other'cholangitic sclerosis'. Radahakrishna Rao

(1935) who studied independently the morbid conditions of the liver in this disease, came to the conclusion that it was one of 'subacute toxic cirrhosis' showing biliary inflammatory lesions only occasionally. The tendency to affect children of the same mother suggests to Bhaskara Menon (1931) an antinatal cause acting slowly after birth, and the histological resemblance to congenital syphilitic cirrhosis leads him to suspect a toxin carried by the umbilical vein, an umbilical vein cirrhosis.

Many American and European writers from time to time have drawn our attention to a type of juvenile cirrhosis occurring in successive children of the same mother. Rossele who has reviewed the literature on the pathology of cirrhosis thoroughly, finds evidence for a hereditary familial tendency lacking. When it occurs in a family it frequently affects more than one child. Parkes Weber (1936) suggests an inborn familial tendency to the development of hepatic cirrhosis in infancy and childhood, and classifies the familial incidence as due to either a congenital tissue or organ inferiority of the liver or as an example of hepatic cirrhosis accompanying and probably constituting a part of acknowledged disease of the congenital developmental case.

Moon (1929) has reported two cases of cirrhosis in children whose livers showed histologically in sections stained for bacteria, cocci in pairs in one case, and in the other streptococci, while on culture a pure growth of streptococcus haemolyticus was obtained

from the latter's liver. It is interesting to note in this case that several children in the family had already died of cirrhosis of the liver. Bingel(1907) was convinced that many cases of cirrhosis in children occurr after epidemics of scarlet fever. Folger (1900) has reoprted a case of cirrhosis with enlarged liver in which streptococci were identified. Thomson believes in infectious jaundice in newly born infants as a result of infection from the umbilicus.

According to Moon (1933) the United States of America Mortality Statistics for 1929 show 64 deaths from cirrhosis of the liver among children under fifteen of which 23 were under two years of age. Though the exact figures for the different types of cirrhosis in children were not available, he found that in the majority of cases under one year of age the cirrhosis was due to congenital obliteration of the bile ducts, and that those occurring after the first year were due to portal or Laennec's cirrhosis. Montgommery and Deaver (1933) Leversen (1908) and Ladd (1928) have cited cases of cirrhosis in infants due to congenital obliteration of the bile duct.

Joseph Calvem and Lionel Saffro have reviewed 36 reported cases of cirrhosis in children accompanied by considerable enlargement of the liver. Autopsy was performed on 17 of which 9 were under two years of age. Of these 6 were hypertrophic cirrhosis, 2 biliary cirrhosis, and 1 sub-acute toxic cirrhosis. Bridgeman and Robertson (1932) Sutton (1930) have reported cases

of atrophic or Laenec's cirrhosis in infancy with hereditary tendency.

Rolleston and MacNee quote (Ponynton and Wyllie) a report of 22 cases of congenital biliary cirrhosis at the Great Ormond Street Hospital for Sick Children during the period 1910 to 1925. Of these cases 15 were due to congenital obliteration of the bile ducts, and 7 were due to other causes. They believe that the obliteration of the bile duct is secondary to the cirrhosis which is of obscure origin. At the Royal Hospital for Sick Children Edinburgh during a period of 14 years, from January 1927 to December 1940 there were 9 cases of cirrhosis of doubtful origin in children under two years, 14 of congenital syphilitic cirrhosis and 3 due to congenital obliteration of the bile ducts.

It can be deduced from the enormous literature available on the subject of cirrhosis of the liver in infants and children that it is not such an uncommon disease either in Europe or in America as one would suspect it to be.

As to the nature of this disease opinion seems to differ. In many of these cases there appears to be an inherent tendency in children of the same mother to be affected by the disease. Syphilis though the commonest and best understood etiological factor has been ruled out in these cases. Infectious diseases and infections of the liver have been the cause of cirrhosis

in a number of cases. Some livers have been classified under the vague and obscure term "Hanot's cirrhosis'. Many were due to congenital obliteration of the bile ducts, and a few to portal or Laennec's cirrhosis and to toxic corrhosis.

A study of the histopathology of the cases from Edinburgh along with those of'Infantile Cirrhosis' of India is undertaken in order to determine if the latter disease is peculiar to India only and if it is not so to find out the possibility of a common etiological factor responsible for these cases.

Table of disorders of the liver from the post-mortem record of the Royal Hospital for Sick Children Edinburgh from Jan. 1927 to Dec. 1940.

Year	P.M.	Cirrhosis of Unknown Etiology	Cong. Syph. Cirr-hos	Cong. Oblit Bile Duct	Acute & S.Acute Atrophy	Icterus Gravis	Other Disorders.
1927	157	1	1		1	1	
1928	146	1	2	1	1		
1929	181					1	
1930	166		1				
1931	149	1	3			1	
1932	157					1	
1933	154	2	2				
1934	127						1.Cent.Necrosis
1935	159	1	1				1.Cholecystitis
1936	149	1	1	1			
1937	193				1		1 Cent.Necrosis
1938	185	1	3			1	2.Fatty Livers.
1939	150			1	3	1	
1940	175	1					
Total 14 Yrs.	2240	9	14	3	6	6	6

Tables showing a summary of the clinical and histo-pathological findings in sixteen cases of cirrhosis of the liver in infants.

Name & Case.No.	Age in Months at Autopsy	Family History	Clinical Abstract.	Feeds.	Inflammatory changes in other viscera	Changes in other organs.	Jaundice.	Ascitis.	Size of Liver.	Hepatic Artery	Portal Vein.	Bile Duct.	Hepatic Vein.	Fibrous Tissue	Parenchyma	Remarks.
V.A.l.	24	Not available	Clay coloured stools anaemia Oedema of lower extremity.	-	-	Spleen enlarged fatty degeneration of kidneys enlarged mesentaric glands.	+ +	+ +	+	Normal	Normal	Bile duct normal Marked proliferation of B.Canaliculi.	Thickened hepatic veins obliteration of central veins. inflam:cell infiltration of the intima.	Insterstitial & interlobular	Degeneration & necrosis of cells.widened lymph space of Disse.A few rounded areas of massive necrosis	Toxic Cirrhosis.
A.ll.	36	Not available		-	-	Enlarged spleen	+	+	— —	Normal	Normal	Periductal fibrosis with inflam:cell in filbration of the periductal region.Proliferation of B. Canaliculi.	Thickened walls obliteration of the lumen of central veins. Infiltration of H.V. with lymphocytes & pigment laden histiocytes.	Increase of F.T. in the Glisson's Cap:Chiefly around the duct.	Regenerating lobules as well as rounded areas of massive necrosis.	Toxic Cirrhosis with perichol-angitis.
B.S. lll.	18	4th child to mother The first three had also died of Infantile B. Cirrhosis children by first wife alive & well		Mothers milk for 1½yrs. Supplemented with cows milk from 3rd.mth. and with rice from 1st.yr.	-	Slight enlargment of spleen	+ +	+ + +	—	Normal	Normal	Duct normal. marked proliferation of Biliary Canaliculi.	Thickening & sclerosis of the wall.	Cellular Interstitial & interlobular. Slight infiltration with round cells.	Both healthy & necrotice cells present pigmented with bile.Lymphatics in portal tract increased in No.& dilated.	Toxic Cirrhosis
B.L. lV.	7	10th child in the family All the other children are healthy.	Anaemia and leucocytosis culture of ascitic fluid sterile.	Mothers milk 6 mths.later supplement by cows milk.	Keratitis & ulceration of mouth.	Spleen enlarged.	+ +	+ + +	+	Normal	Normal	Duct normal marked proliferation of B. Canaliculi.	Thickening of the wall periphlebitis.	Spongy connective tissue.Interstitial & interlobular.	Oedematous distension of lymph space of Disse.Degeneration & necrosis pigmented with bile.	Toxic Cirrhosis.

Continued

Name & Case No.	Age in Months at Autopsy	Family History	Clinical Abstract	Feeds.	Inflammatory changes in other Viscera.	Changes in other organs.	Jaundice.	Ascitis.	Size of Liver	Hepatic Artery	Portal Vein	Bile Duct.	Hepatic Vein.	Fibrous Tissue	Parenchyma	Remarks.
B.V. V.	16	All the other children are healthy					+	+	−	Normal	Normal	Duct normal proliferation of B.Canaliculi.	Considerable thickening of the wall. Infiltration with scanty lymphocytes & plasma cells.	Cellular in type chiefly interstitial. scanty round cell infiltration.	Diffuse degeneration changes & a few areas of massive necrosis	Toxic Cirrhosis.
P.V1.	20	Not available					+	+	+	Normal	Normal	Duct normal slight proliferation of B. Canaliculi.	Endophlebitis with intimal thickening of the larger veins & sclerosis of the terminals Infiltration with lymphocytes & plasma cells.	Intercellular & interlobular.	Degenerative changes of the cells & foci of necrosis	Toxic Cirrhosis.
J.C. V11.	1¼	The younger sister died of same disease later.	Distended superficial abdominal veins.	Cow's milk & S. Laura's food.	Commencing Pertitonitis	Spleen slightly enlarged. Haemorrhage into the tubules of the kidney.	++	+++	+	Normal	Normal	Duct normal great proliferation of Canaliculi.	Slight increase in thickness inflam: cellinfiltration of the wall.	Interstitial distribution.	Massive necrosis of liver cells & regeneration of lobules.	Toxic Cirrhosis

Continued

Name & Case.No.	Age in Months at Autopsy	Family History.	Clinical Abstract.	Feeds.	Inflammatory changes in other viscera.	Changes in other Organs.	Jaundice.	Ascitis.	Size of Liver.	Hepatic Artery.	Portal Vein.	Bile Duct.	Hepatic Vein.	Fibrous Tissue.
M.C. VIII.	5	The elder brother died of the same disease.	Changed from breast because not gaining weight. Gained rapidly on change to L.Acid milk.	Breast fed for 9 wks. Later supplemented by Virol the lactic acid milk.	-	Adenoma of Rt.Sup.renal spleen. enlarged firm.	+	—	Normal	Normal	Normal	Duct normal. Moderate proliferation of B.Canaliculi.	Thickening and sclerosis of the hepatic venous tree.	Interstitial.
J.W.1. IX.	1½	8 children 1 died of convulsion at 6 weeks 1 of cirrhosis at 9 wks.			Terminal acute peritonitis.	Spleen enlarged.	+	+++	—	Normal	Normal	Duct normal slight proliferation of B. Canaliculi.	Inflam:cell infil of H.V. with narrowing of lumen thickened central vein.	Interstitial increase of F.Tissue slight increase in Portal Trac also.
J.W.2. X.	2¾	8 children 1 died of convulsion at 6 weeks 1 died of Jaundice cirrhosis at 6 weeks.	Motions clay coloured and green. Some Oedema of lego.	On breast for a few days only. Then dil. milk.	-	Spleen slightly enlarged Hyperaemia of pulp thickening of capsule and trabecular	+ +	+ +	—	Normal	Normal	Duct normal. No proliferation of B. Canaliculi.	Inflam:cell infiltration marked thickening of H.V. and central vein.	Interstitial fibros with slight increase of F.T. in the Portal Tract.
C.T. XI.	1¾		Bleeding from umbilicus when cord separated.	Cows milk.	Broncho-pneumonia.	Spleen enlarged.	+ + +	—	+	Normal	Normal	Duct normal very slight proliferation of ducts.	Moderate thickening of the walls of the Hepatic Venous tree. Slight inflam:cell infiltration.	Interstitial fibros type extending both from P.Trac &.H.V. into the lobule.
M.O'N. XII.	10	Premature birth by two months.	Clinical bio-chemical and X.Ray evidence of active rickets At 6 month catarrhal Jaundice.	On mothers milk for 1 month only. Later artificial.	Broncho-pneumonia.	SPleen much enlarged.	—	—	—	Normal	Normal	Duct normalslight proliferation of bile canaliculi.	Normal.	Narrow band of fibrous tissue lining portal tracts and hepatic ve

Organs.	Jaundice.	Ascitis.	Size of Liver.	Hepatic Artery.	Portal Vein.	Bile Duct.	Hepatic Vein.	Fibrous Tissue.	Parenchyma.	Remarks.
p.renal firm.	+	—	Normal	Normal	Normal	Duct normal.Moderate proliferation of B.Canaliculi.	Thickening and sclerosis of the hepatic venous tree.	Interstitial.	Extensive necrosis and falty degen of liver cells.Round cell infiltration	Acute liver atrophy superimposed in slowly progessing toxic cirrhosis.
.	+	+ + +	—	Normal	Normal	Duct normal slight proliferation of B. Canaliculi.	Inflam:cell infil of H.V.with narrowing of lumen thickened central vein.	Interstitial increase of F.Tissue slight increase in Portal Tracts also.	Necrosis of liver cells around cental vein.	Toxic Cirrhosis.
enlarged ulp thickle and tra-	+ +	+ +	—	Normal	Normal	Duct normal.No proliferation of B. Canaliculi.	Inflam:cell infiltration marked thickening of H.V. and central vein.	Interstitial fibrosis with slight increase of F.T. in the Portal Tract.	Necrosis of single cells and small groups of cell.	Toxic cirrhosis.
.	+ + +	—	+	Normal	Normal	Duct normal very slight proliferation of ducts.	Moderate thickening of the walls of the Hepatic Venous tree.Slight inflam:cell infiltration.	Interstitial fibrosis type extending both from P.Tracts &.H.V.into the lobule.	Diffuse and scattered necrosis mostly adjoining the central vein liver cell pigmented with bile.	Toxic Cirrhosis.
arged.	—	—	—	Normal	Normal	Duct normalslight proliferation of bile canaliculi.	Normal.	Narrow bands of fibrous tissue linking portal tracts and hepatic veins.	Extensive fatty change of most of the liver cells.	Portal Cirrhosis.

Continued

Name & Case No.	Age in Months at Autopsy.	Family History.	Clinical Abstract.	Feeds.	Inflammatory changes in other Viscera.	Changes in other Organs.	Jaundice.	Ascitis.	Size of Liver.	Hepatic Artery.	Portal Vein.	Bile Duct.	Hepatic Vein.	Fibrous Tissue.
E.M. XIII.	3	8 children alive. 1 died of T.B. meningitis and 1 of Broncho-Pneumonia.	Glycosurea for 14 days before death.	Artificial	-	Spleen was much enlarged. Haemorrhage in the Pancreas.	-	-	+++	Normal	Normal	Normal. Mod: proliferation & lengthening of B. Canaliculi.	Normal.	Increase in Portal tracts fibrous ban extend to adjacent P. tracts.
J.N. XIV.	3½	5 children 2,3, & 4 week during infancy.	Urine bile stained 1 week green motions.	Breast feeding	Sepsis at umbilicus.	Spleen much enlarged pulp empty.	++	+	+ +	Normal	Normal	Normal	Normal	Early collagenous change of reticul of sinusoids with out apparent increase of fibrobl
W.A. XV.	¾	2nd child died 4 yrs. ago at birth.	Blood cult sterile. Diarrhoea 7 days. Extreme aganosis.	Breast fed.	Bilateral corneal ulceration No G.C. Morax auxeufeld Bacillus.	Meninges congested Lungs were congested and oedemates.	-	-	Normal	Periartirites with considerable narrowing of lumen.	Thickened lumen narrowed.	Periductal inflam: cell infiltration & periductal fibrosis. Proliferation of Canaliculi.	Very light thickening of the hepatic venous tree.	Increase of fibro tissue localised portal tract espe ially periductal distribution.
T.P. XVI.	8	1st child.	Motions clay coloured from birth.	Breast fed few days only cow's milk & S.Laura's.	-	Pulmonary Oedema.	+++	+++	+	Lumeu narrowed periarteritis.	Lumen narrowed Periphlebitis.	Obliteration of the lumen of Ducts marked proliferation of B. Canaliculi.	Normal.	Chiefly in the Cl sson's Capsule. R also inter-cellul

atory in other .	Changes in other Organs.	Jaundice.	Ascitis.	Size of Liver.	Hepatic Artery.	Portal Vein.	Bile Duct.	Hepatic Vein.	Fibrous Tissue.	Parenchyma.	Remarks.
	Spleen was much enlarged.Haemorrhage in the Pancreas.	—	—	+ + +	Normal	Normal	Normal.Mod:proliferation & lengthening of B. Canaliculi.	Normal.	Increase in Portal tracts fibrous bands extend to adjacent P.tracts.	Marked fatty change and very little necrosis.	Portal Cirrhosis.
is ilicus.	Spleen much enlarged pulp empty.	+ +	+	+ +	Normal	Normal	Normal	Normal	Early collagenous change of reticulum of sinusoids with out apparent increase of fibroblasts	Cells appear foamy cell outlines well marked.Some show degenerative changes collapse of sinusoids and distension of lymphspace of Disse.	Serous Hepatitis.
ral corneal tion No G.C. auxeufeld us.	Meninges congested Lungs were congested and oedemates.	—	—	Normal	Periartirites with considerable narrowing of lumen.	Thickened lumen narrowed.	Periductal inflam: cell infiltration & periductal fibrosis.Proliferation of Canaliculi.	Very light thickening of the hepatic venous tree.	Increase of fibrous tissue localised to portal tract especially periductal in distribution.	Extreme fatty degeneration.Nuclei of cells well stained.	Early biliary cirrhosis of cholangitic type.
	Pulmonary Oedema.	+ + +	+ + +	+	Lumeu narrowed periarteritis.	Lumen narrowed Periphlebitis.	Obliteration of the lumen of Ducts marked proliferation of B.Canaliculi.	Normal.	Chiefly in the Glisson's Capsule.But also inter-cellular.	Multi nucleated regenerating liver cells.	Cirrhosis due to congenital obliteration of Bile Duct. Cholostatic Biliary Cirrhosis.

MATERIAL and METHODS of STUDY.

The material for study consisted of sections from sixteen cases; six from India and ten from the Royal Hospital for Sick Children, Edinburgh. Though the mortality rate for 'Infantile biliary cirrhosis' is great in Calcutta (700 deaths or more per year) and Madras, the scarcity of post-mortem material of these cases is explained by the fact that most parents either out of sentimental reasons, or due to religious scruples do not give their consent for post-mortem. Of the six cases available, two were obtained from the Pathology Department of the Vizagaptam Medical College through the kind permission of Prof. Bhaskara Menon, and the other four were supplied by Prof. Ramachandra Rao of the Madras Medical College. These sections were fixed in formalin, and preserved in glycerine and water.

Of the ten cases from The Royal Hospital for Sick Children, Edinburgh one is an authentic case of Congenital Obliteration of bile duct, and the other nine cases of cirrhosis of obscure etiology in children under two years of age. Paraffin blocks for eight of these cases and sections of the liver and other organs stained with haematoxylin and eosin in the case of the remaining two were placed at my disposal by Dr MacGregor.

Staining methods used.

(1) For routine staining. Meyers acid alum haematoxylin and eosin.

(2) For connective tissue. Heidenhain's Azan method.

(3) Verheoff's elastic tissue stain.

(4) Sudan III. counter stained with haematoxylin for fat.

(5) Dobell's modification of Levatidi for Spirochaetes.

(6) Koppeleoff and Beirmann's modification of grams method for bacteria.

(7) Foot and Ménard's technique for reticulum.

Six of the sections from India which were preserved in glycerine and water were washed in water for 48 hours and post fixed in Helly's fluid before embedding in paraffin.

Cytology of the liver and some facts bearing on its pathology.

The parenchyma consists of hepatic trabecula with a potential axial lumen which is continued at the periphery as the biliary afferent or the canal of Hering. There is cross anastomosis between the hepatic cords and they form a continuous mantle between and around the sinusoids.

Vascular pattern. The terminals of the hepatic and the portal veins are formed after dividing five or six times at right angles to the main trunks. There is a symmetrical distribution of the portal and hepatic venous trees between the so-called lobules, so that two hepatic terminals are placed on either side of a

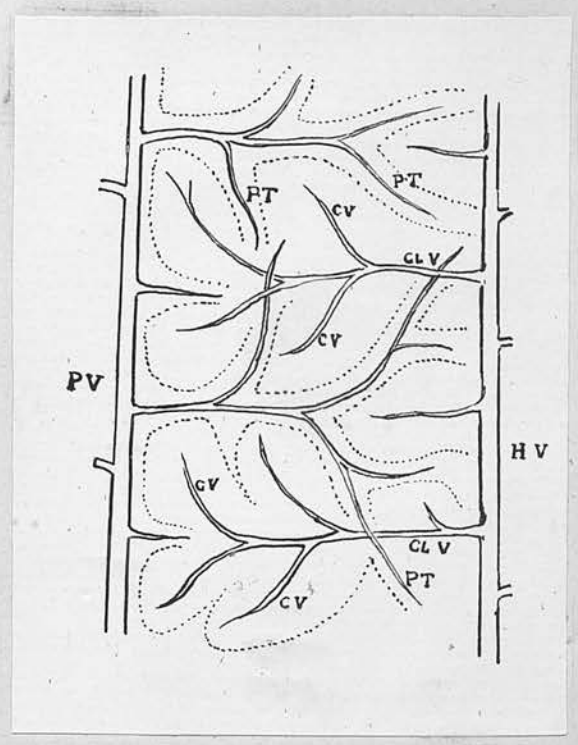

Fig. I. A diagramatic representation of the hepatic and portal venous systems of the liver.

C.V. Central vein, C.L.V. Collecting vein, H.V. Large branch of the hepatic vein, P.T. Portal terminal.
P.V. Large branch of the portal vein.

(From F.P. Mall. AM.J.Anat. 1906 Vol. 5).

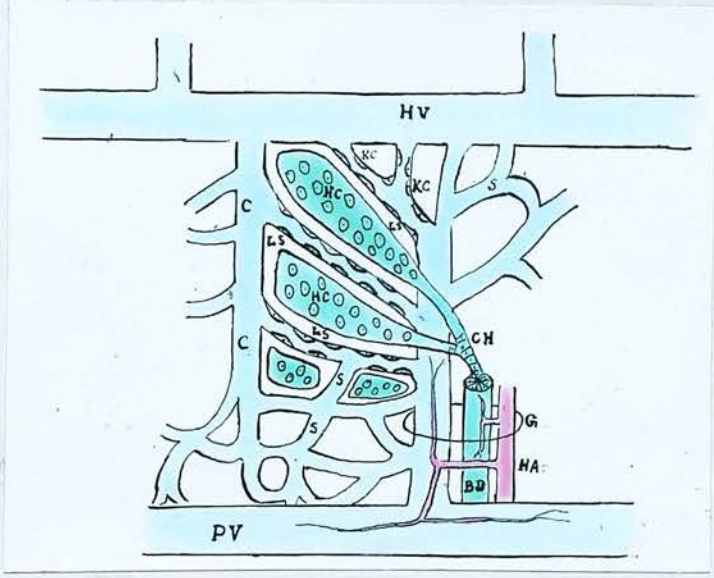

Fig II. A diagram representing the histology of the liver.

G. Glisson's Sheath, H.A. Hepatic Artery, P.V. Portal vein, B.D. Bile duct, C.H. Canal of Hering.
H.C. Hepatic cord, S. Sinusoids.
C. Central vein, H.V. Collecting vein,
L.S. Lymph space of Disse,
K.C. Kupffer cells.

(From Ramachandra Rao V.M.C.M. 1932)

portal terminal at a constant interval (Cowdry). Actually the appearance of a lobular structure in the parenchyma is due to this architectural pattern of its vascular trees and their tributaries.

Hepatic artery The distribution of the hepatic artery is chiefly to the Glisson's sheath, and the structures therein. According to Cameron and Mayes, the hepatic artery breaks up in to a plexus of capillaries distributed mainly to the bile ducts, and the branches of the portal vein. From this plexus blood is collected in to venules which drain in to the portal venules, and thence into the sinusoids. Direct communication between the hepatic artery and portal vein was not found; but it is maintained that some capillaries do pass directly from the smaller branches of the hepatic artery into the sinusoids.

The presence of a perisinusoidal lymph space between the hepatic cords and the wall of the sinusoids has been recognised by Kupffer, MacGillavry, Disse, and others. These are said to communicate with the lymph plexus of the glissons sheath.

Case No. 1.

Name - V.A. Female infant. Aged 2 years.

Post-mortem

Morbid Anatomy Smooth capsular surface. Edges sharp. Uniform bile staining of the parenchyma on section. Sclerotic thickening of the larger portal spaces.

Histology The parenchyma is permeated with irregular bands of wavy fibrous tissue isolating the hepatic cords into groups of varying size. In places the collagen fibres have insinuated themselves in between individual liver cells giving the whole field the appearance of interstitial cirrhosis. The parenchymal cords are widely separated by the gaping sinusoids and many of them show degenerative changes and vacuolation. The nuclei are faintly stained, the cytoplasm granular and eosinophilic. The potential lumen in the hepatic cords is filled in places by a plug of inspissated bile. In sections from different parts of the liver the microscopical changes are slightly different; the islands of regenerated liver cells being larger and degeneration less pronounced. Here and there among the degenerated liver cells oval or rounded islands of parenchymal cells are found to be in various stages of necrosis. The healthy cells around such islands are compressed and stretched over them, a condition suggesting an oedema of the involved cells prior to their disintegration. There is moderate infiltration with polymorphs and lymphocytes in the necrotic debris. The highly resistant biliary canaliculi (canal of Hering) are in the process of proliferation.

Bile ducts Proliferation of biliary canaliculi varies in different sections. Numerous long and tortuous or short and tubular bile ducts are embedded in thick wide bands of cellular fibrous tissue. In

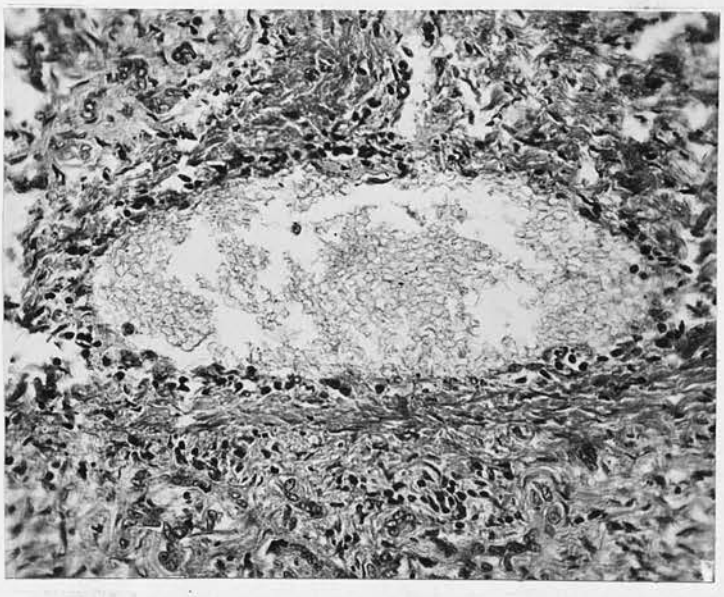

Fig. III, Liver of Case I. A branch of the hepatic vein showing hyperplastic endophlebitis. H and E. x 275.

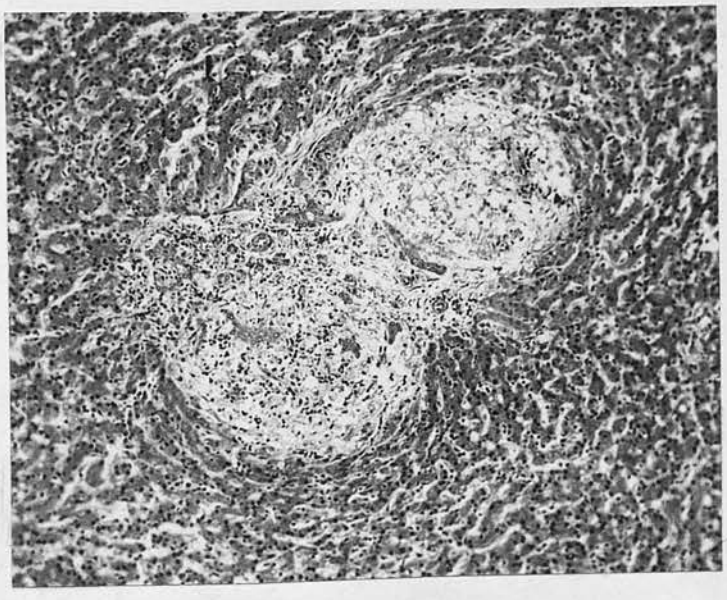

Fig IV. Liver of Case I. Massive necrosis of liver cells. The healthy cells are compressed and stretched out over the islands of necrosis. There is moderate infiltration with lymphocytes and polymorphs.

H and E x 85.

other parts the fibrous tissue bands are devoid of any proliferated bile ducts. The ducts in the portal tract are unaffected, their lining epithelium being healthy and there is absence of any infilltration with inflammatory cells. A few of the proliferated bile ducts contain inspissated bile, showing a direct continuity with healthy liver cells.

Hepatic Vein Branches of the hepatic vein are scarcely to be seen in the section, but wherever they are found they are seen to be surrounded by dense fibrous tissue and their walls are thick and sclerosed. A few of the larger branches show endophlebitis and infiltration of the intima with inflammatory cells. Hepatic terminals are scarce, their walls being involved in similar sclerosis with obliteration of the lumen. The parenchymal cells around such central veins have completely disappeared, being replaced by cellular fibrous tissue.

The Portal tracts. There is no marked increase of fibrous tissue in the glissons capsule. There is no periductal sclerosis. The hepatic artery and portal vein show no noticeable change. There is slight fibrosis of the periphery of the lobules in the region adjacent to the portal tract which tends to magnify the apparent width of the tract.

Case No. 2.

Name A.

A Hindu female child aged 3 years.

Morphology. Liver markedly contracted with an uneven nodular surface and a sharp edge. The cut surface was slightly bile stained. The portal spaces were wide, greyish white and sclerotic. The branches of the hepatic veins were surrounded by wide bands of connective tissue.

Histology.

Parenchyma. The normal architecture of the liver is completely lost. The formation of Pseudo-lobulations of parenchyma from the proliferation of surviving liver cells in the midst of collapsed lobules has upset the whole lobular pattern. Central veins are scarce. Sinusoids are wide and empty in the pseudo-lobules. The Kupffer cells show elongation of their nuclei (the primary change is the transformation of the Kupffer cells into fibroblasts) No pigment can be seen in these cells. The liver cells in the pseudo-lobules are fairly well preserved, the nuclei taking the normal nuclear stain, but many of the cells at the periphery of the lobules show degenerative changes their nuclei undergoing Karyorrhexis. Mitosis is scarcely to be seen. A few scattered rounded areas of massive necrosis of parenchymal cells can be seen where the bare outline of the liver cells are made out or where the liver cells have completely disappeared leaving a loose frame work of reticulum in

which are numerous ~~tortuous bile ducts separate~~ polymorphs, swollen Kupffer cells and persisting bile ducts in varying degrees of proliferation. Some areas where there had been an early necrosis now show numerous tortuous bile ducts separated by cellular fibrous tissue. A few polymorphs are also present.

Bile ducts. There is a marked periductal fibrosis in the Glissons Sheath, but in no case is the lumen of the duct obliterated. Some of the ducts are filled by plugs of bile thrombi but this is not a marked characteristic. There is a slight infiltration of periductal fibrous tissue with lymphocytes indicating a chronic inflammatory change in the wall of the bile duct. There is no desquamation of the lining epithelial cells and the cells themselves are normal and well stained. Besides the numerous tortuous bile ducts observed in the necrosed lobules, many proliferating bile ducts are also present in the meshes of the fibrous tissue in the Glissons capsule as well as in that encircling the newly formed liver lobules. A rare duct here and there show inspissated bile in its lumen.

Portal tract. There is a marked increase in the fibrous tissue. The hepatic artery and portal vein show no apparent change. The fibrous tissue is seen chiefly around the bile ducts.

Bands of fibrous tissue extending between portal tracts and the sclerosed hepatic veins contain in the interstices numerous proliferated bile ducts and attenuated hepatic cords.

There is great thickening and sclerosis of the hepatic venous tree and this change has affected the central veins also which show a diminution and an obliteration of the lumen. The walls of many of the bigger divisions of the hepatic vein are infiltrated with lymphocytes and pigment laden histiocytes.

Case No. 3

Name - B.S. Male infant. Aged 18 months.

Morbid anatomy. Surface of liver uneven and granular. Cut section shows yellowish rounded areas of liver parenchyma in a matrix of pale pink fibrous tissue. Section bile stained. Liver smaller than normal.

Histology The parenchymal cells are seen as rounded islands of varying size in a matrix of cellular connective tissue which has condensed in concentric laminae around the different lobules. The cells of many of these lobules are well preserved; but there are some lobules where the whole mass of cells are vacuolated granular and necrotic. In others the cells in the centre of the lobule are bile stained, granular, with indistinct cell outline and nuclei which are not stained. In between the cells of the hepatic cord lie plugs of inspissated bile.

Bile ducts. Proliferation of bile ducts is a marked feature. They are seen as either short ducts or long tortuous tubules in the meshes of the dense fibrous tissue, and consist of small cubical cells disposed in a double row of parallely placed cells. Branching of

such tubules are occasionally seen.

Glissons Sheath does not show any increase in the connective tissue, and the bile duct, hepatic artery and portal vein are normal. The lumen of the bile ducts are patent and the epithelium intact; but some of them contain plugs of inspissated bile.

Branches of the hepatic vein and their terminals are not obvious under the microscope except with specific stain for fibrous tissue; but a few of the larger ones which can be made out show enormous thickening of their wall leading almost to complete occlusion of their lumen, and some of the smaller ones have undergone complete obliteration.

The connective tissue which breaks up the parenchyma into lobules, and into groups of a few cells is abundant and highly cellular. It is infiltrated with lymphocytes and endothelial cells.

The lymphatics in the portal spaces, and in the newly condensed fibrous tissue is greatly increased in number and in the width of their lumen.

Case No. 4.

Name B.L. Female infant aged seven months.

Morbid Anatomy. Liver moderately enlarged. Surface finely granular, and consistency firm, but section bile stained. Fine bands of fibrous tissue form a net work in the meshes of which are small areas of yellowish liver cells.

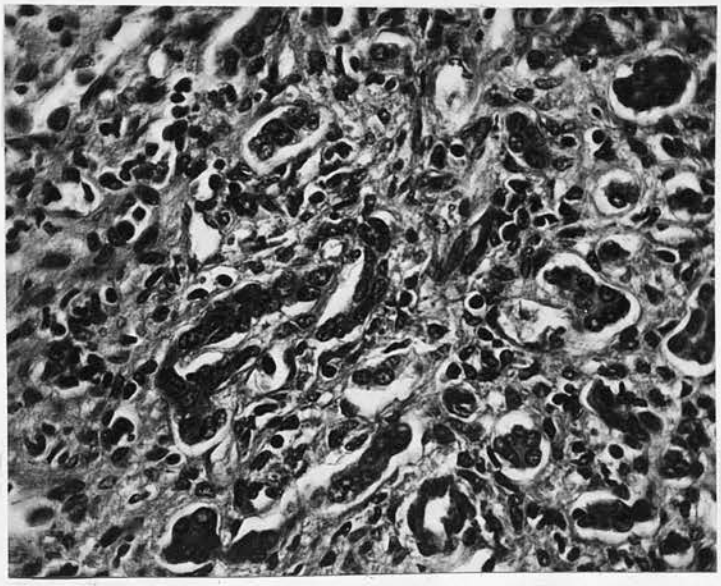

Fig V. Liver of Case III. Numerous proliferated bile ducts are seen in the meshes of dense cellular fibrous tissue.

H and E x 300.

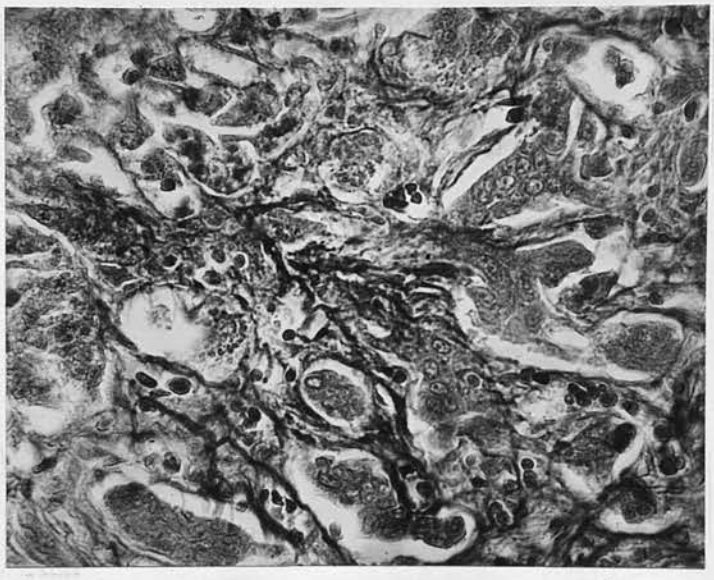

Fig. VI. Liver of Case IV. Showing fine strands of fibrous tissue between hepatic cords and distension of the lymph space of Disse.

Azan x 300

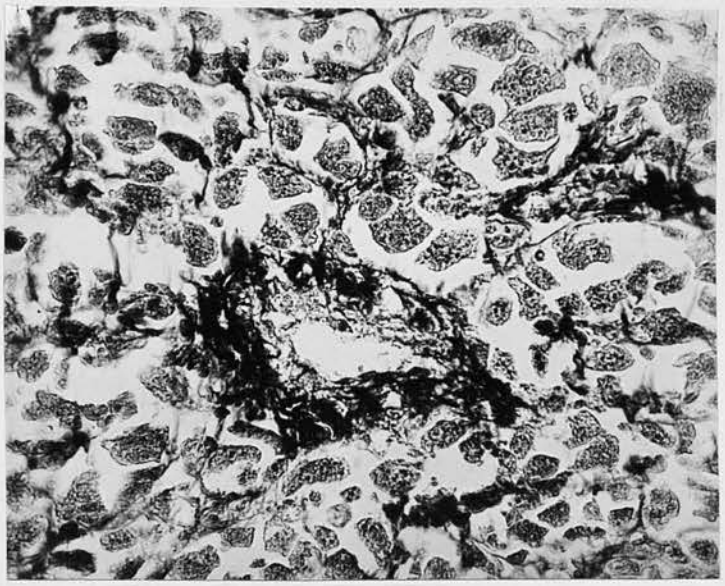

Fig. VII. Liver of Case IV. Showing a marked thickening of the central vein and narrowing of the lumen. The lymph spaces are distended. The parenchymal cells are shrunken, irregular in shape with granular cytoplasm.

Azan x 275.

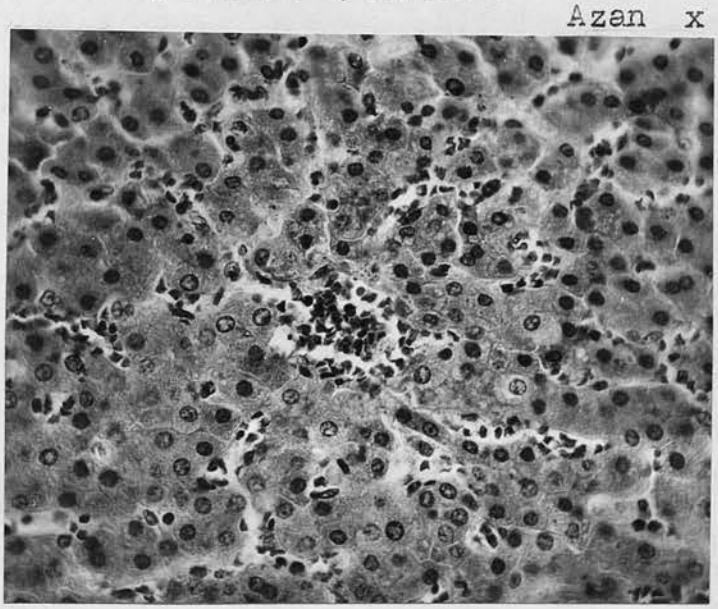

Fig. VIII. Liver of a healthy child aged 7 months. No fibrous tissue can be seen in the wall of the central vein. The lumen is wide and contains numerous red cells. Sinusoids are moderately open and contain red cells. Lymph space of Disse is not seen.

H and E x 350.

Microscopic Anatomy. The hepatic cells are broken up into small clusters and into individual cells by fine strands of cellular connective tissue. The cells show varying stages of degeneration and necrosis ; a large proportion of them having a granular cytoplasm containing particles of bile pigment, and with nuclei which are ill stained or not at all. The parenchymal cords are widely separated as a result of oedematous distension of the lymph space of Disse. A few broad bands of rather spongy connective tissue link up the neighbouring portal spaces to thickened branches of hepatic veins, and in this are observed numerous newly formed tortuous and branching bile canaliculi. The Glissons sheath show an increase in connective tissue. The hepatic artery, portal vein, and bile duct are normal. The hepatic veins show a moderate thickening of the wall but there is no endophlebitis or occlusion of the lumen.

The Connective Tissue occurs as delicate strands of fibrous tissue, permeating in between the cords of parenchymal cells separating them into smaller segments, and destroying the lobular pattern of the liver completely. The resulting cirrhosis is inter cellular in character suggesting syphilitic cirrhosis, but the absence of perivascular cuffing with lymphocytes and plasma cells, and the absence of miliary syphiloma excludes any such possibility.

Case No. 5.

Name B.V. Male child aged 16 months.

Morphology. Surface uneven and finely granular. The thickened hepatic veins appear prominent on the cut surface. Islands of pale yellowish parenchyma in size varying from pin point to that of a millet seed are uniformly distributed in a network of sclerosing fibrous tissue.

Histology. The presence of a loose cellular fibrous tissue in between groups of hepatic cords and single cells has deranged the normal lobulations. The lobules are devoid of central vein. The cytoplasm of the cells appear granular and eosinophilic and many of the nuclei fail to stain. A few isolated oval areas show massive necrosis of the parenchyma with infiltration of polymorphs and lymphocytes, and a proliferation of surviving biliary canaliculi (canal of Hering). No mitosis or double nuclei in the cells as an evidence of regeneration can be seen.
The Glissons sheath and the structures in it appear normal. There is no periductal fibrosis. The artery and vein are healthy.

Hepatic veins and their smaller subdivisions show considerable thickening of their walls with an apparent diminution of their lumen. Many of them show a lymphocyte and plasma cell infiltration of their wall. The hepatic terminals of the central veins having undergone complete obliteration are absent in most of the lobules.

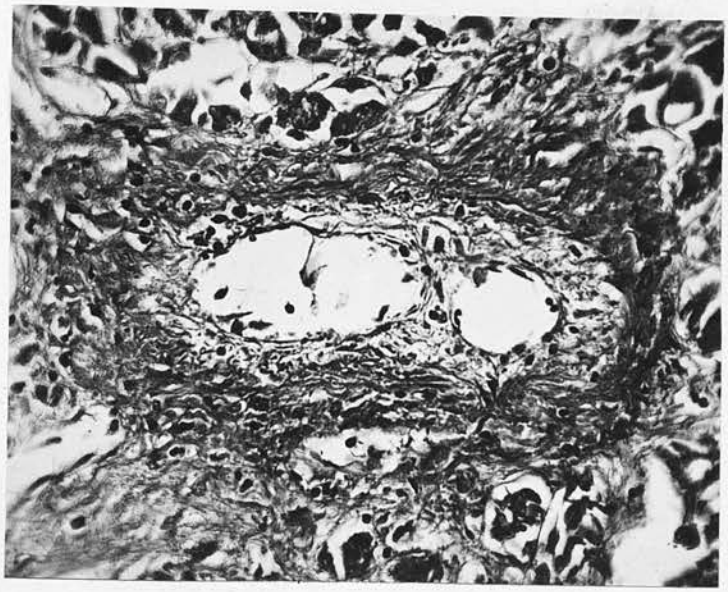

Fig IX. Liver of Case V. Showing marked thickening of the wall of a hepatic vein and partial obliteration of its lumen.

H and E x 235.

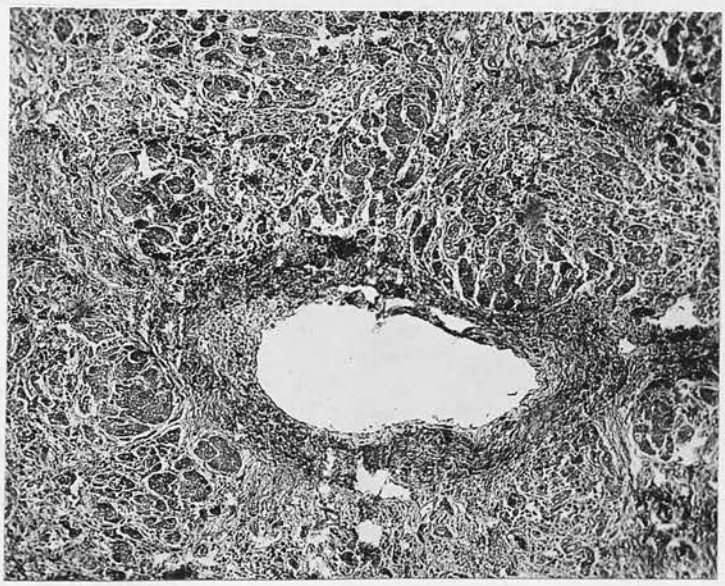

Fig X. Liver of Case VI. A large branch of the hepatic vein showing thickening of the wall. The connective tissue of the intima is swollen and has encroached on the lumen of the vein. Interstitial type of fibrosis seen in the surrounding area.

Azan x 70.

Branched and unbranched biliary canaliculi as well as compressed hepatic cords are observed in large numbers in the interstices between the fibrous tissue that is abundantly present. There is a scanty infiltration of the fibrous tissue with round cells. The sinusoids are dilated, and the Kupffer cells contain granular bile pigment

Case No. 6.

Name. P. Hindu male child, aged 20 months.

Morphology. Surface uneven and coarsely granular. Cut section is bile stained capsule is thickened and the hepatic radicles stand out with thickened walls. The parenchyma is seen as crescentic or oval yellowish homogenous areas on a back ground of bile stained, firm connective tissue.

Histology. The histological picture is similar to those examined before in that the disposition of the fibrous tissue is interlobular and intercellular. The cells are degenerated and in places necrotic. The areas where necrosis had occurred previously show condensation of the reticulum, and collagenous transformation of the fibres.

The portal tracts are normal, the artery, vein, and duct being well preserved. Bile duct proliferation is slight.

The hepatic venous radicles show remarkable thickening of their wall which has caused considerable

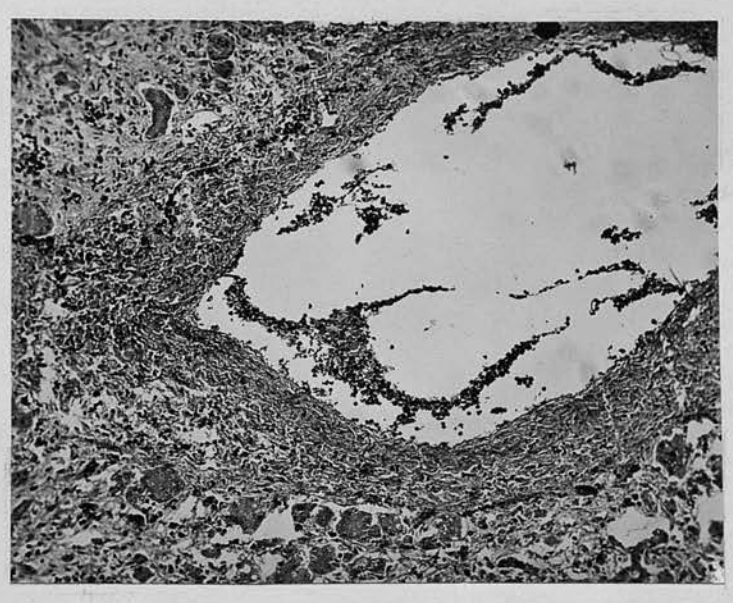

Fig XI. Liver of Case VII. A large hepatic vein showing diffuse inflammatory cell infiltration of the wall is seen. The parenchyma around the vessel have mostly disappeared and has been replaced by fibrous tissue.

H and E x 80.

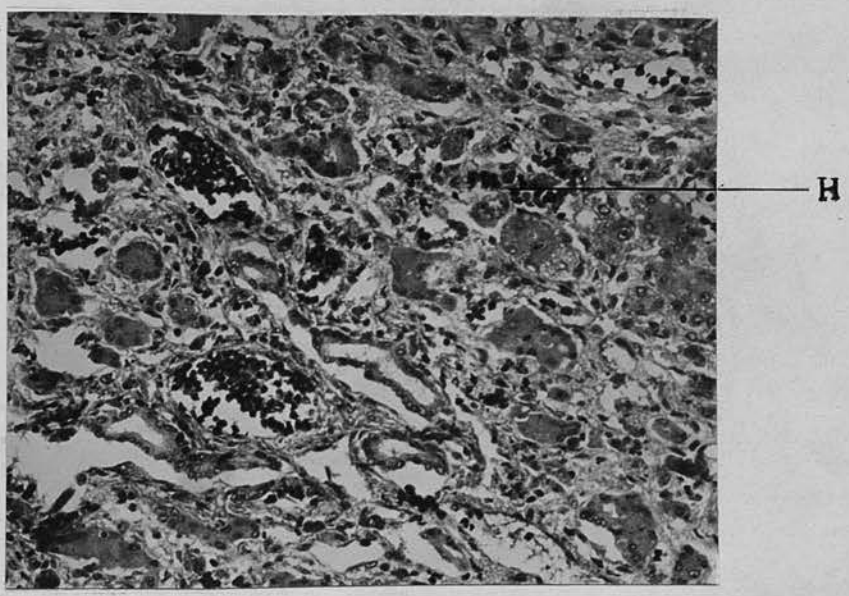

Fig. XII. Liver of Case VII. Showing a moderate proliferation of biliary canaliculi in a dense net work of fibrous tissue.
H. Islands of persistent haemopoesis.

H and E x 200.

granular, vacuolated and invariably shows the-presence of bile pigment. Here and there masses of liver cells are undergoing necrosis, their cytoplasm disintigrating into granular bile stained debris and their nuclei showing karyorrhexis and lysis, resulting in the collapse of the sinusoidal reticulum. In these areas there is great proliferation of biliary canaliculi, which vary in their character from structures closely resembling hepatic cords to those of fully developed biliary afferent. Plugs of inspissated bile is found in the hepatic cords as well as in some of the newly formed bile canaliculi. The portal tracts do not show any definite increase in fibrous tissue, or exudate of inflammatory cells.

The epithelium of the bile duct is intact and the lumen patent. The branches of the hepatic vein show slight fibrosis of their wall and there is slight but apparent infiltration of their wall by lymphocytes endotheloid cells and very few polymorphs. Coarse granular pigment is observed in the Kupffer cells of the sinusoid, in the histiocytes and in isolated liver cells embedded in the fibrous tissue.

Case No. 8.

Name. M.C. Female infant aged 5 weeks.

Morbid Anatomy of Liver.

Size normal. It was abnormally hard with sharp edges. Surface smooth, pale yellowin colour, irregularly

mottled with red. A few flat nodules of light yellow colour were noticeable on the surface. Moderately tough to cut. Cut section showed the same type of mottling as on the surface; but patches of fibrous tissue were noticed giving the cut section a speckled appearance. A few circular yellow patches suggestive of regenerating liver tissue were present in the cut surface.

Microscopic Appearance.

Parenchyma. There is extensive necrosis and fatty degeneration of the liver cells throughout the lobule except for a narrow margin of cells around the portal tract. The cytoplasm is eosinophilic, granular and vacuolated, and the nuclei are pyknotic and show karyorrhexis. The sinusoids are distended with red cells, many polymorphs, lymphocytes and monocytes . In those areas where the necrotic process has far advanced, the sinusoids have collapsed through compression by the oedematous necrotic debris and here infiltration with inflammatory cells is well seen. The Kupffer cells are swollen and the nuclei are polymorphic. Many of these are impregnated with bile pigment.

The lymph space of Disse shows oedematous distension with attenuation of the hepatic cord. Portal tracts show increase in fibrous tissue, but fine collagenous strands of connective tissue are seen to extend into the neighbouring lobules along the walls of the sinusoids, evidently due to the transformation of the

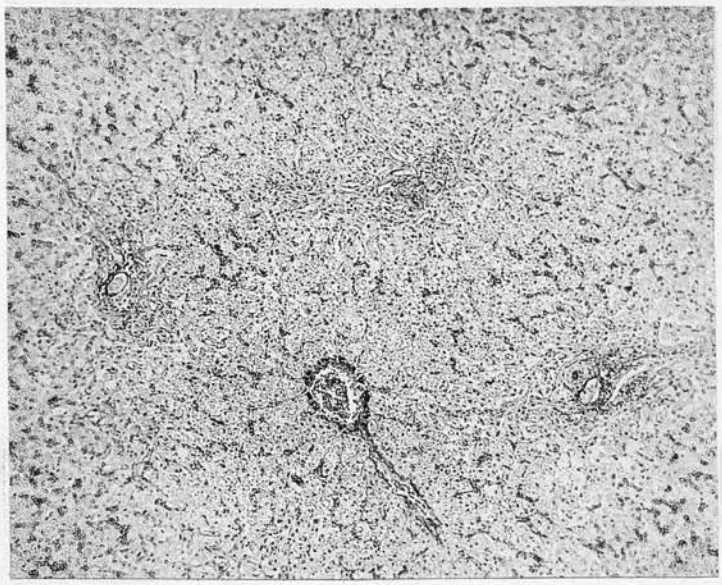

Fig XIII. Liver of Case VIII. A central vein with marked thickening and sclerosis of its wall is seen. The three portal terminals which are observed in the same field are normal. There is a slight increase of fibrous tissue around the Gilsson's Sheath.
Azan x 70.

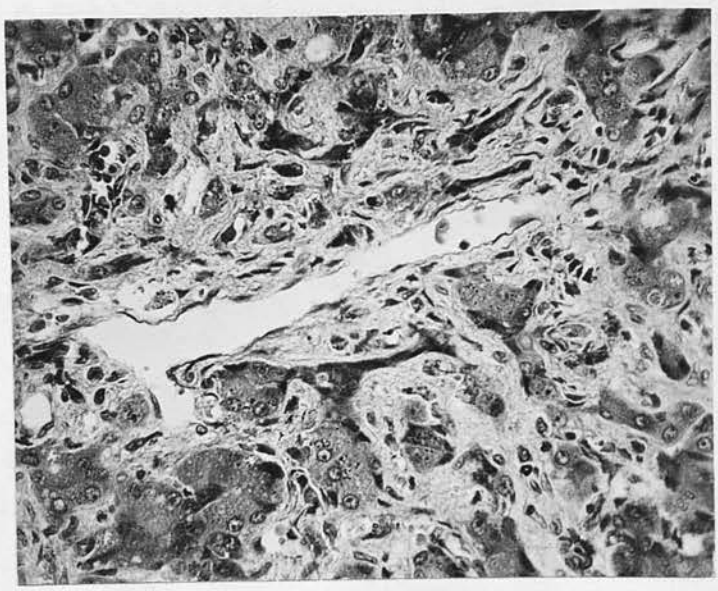

Fig. XIV. Liver of Case IX. Showing a longitudinal section of a central vein with marked thickening of its wall. The parenchyma around it has been replaced by dense fibrous tissue. The hepatic cords are attenuated.
H and E x 275.

reticulum fibres into collagenous strands.

The portal vein, hepatic artery and bile duct are normal. There is some infiltration with polymorphs, lymphocytes and eosinophiles but this does not bear any relation to the artery, vein or duct, and appears to be part of the general cell mobilization which has affected the whole organ. There is moderate proliferation of biliary canaliculi in the neighbourhood of the portal tract. The cytoplasm and nuclei of these epithelial cells take the stain well.

The sinusoidal bed around the hepatic terminals are also involved in the collagenous transformation of its reticular wall which has resulted in a fine type of interstitial cirrhosis. The hepatic venous tree and its terminal branches show thickening and sclerosis of their wall but the lumen is patent, and widely dilated. The general picture is that of acute liver atrophy in a liver which shows evidence of slowly progressing toxic cirrhosis.

Case No. 9.

Name. J. W. Male infant, aged six weeks.

Macroscopic appearance. Liver slightly smaller than normal. Colour deep green, surface finely granular, and consistency firm. It was rather tough to cut and the cut surface suggested fine monolobular cirrhosis. Gall bladder normal. Cystic and common bile duct were patent.

Microscopic Appearance. The parenchyma is broken up into narrow tortuous columns and into small groups of cells by cellular and dense connective tissue. The normal lobular pattern is completely lost. Here and there small clumps of cells surrounding the thickened central vein have undergone necrosis, and the transformation of the sinusoids into capillaries can be made out. The parenchymal cells are loaded with coarse and fine granules of greenish yellow bile pigment. Plugs of bile thrombi fill up the potential lumen in the hepatic cords. The out-line of the cells is indistinct and the cytoplasm granular and vacuolated. Mitosis of cells and regeneration of lobules of liver cells are absent.

Portal tract. There is moderate increase in the fibrous tissue of the portal tract. The hepatic artery and portal vein are normal. The bile ducts are healthy their lumen being patent and the lining epithelial cell intact and normal. There is some proliferation of the biliary canaliculi in the region adjacent to the portal tract and a few of them are distended with plugs of inspissated bile.

Kupffer cells show moderate proliferation. Many of them contain fine granules of bile pigment in their cytoplasm, while in others the nuclei are elongated and spindle-shaped and represents a gradual transformation of these cells into fibroblasts.

There is much thickening of the walls of hepatic

venous tree with infiltration of inflammatory cells in some branches, resulting in considerable narrowing of the lumen. The central veins are also thickened and sclerosed and an endophlebitis is a recognisable feature in most of them.

Case No. 10.

Name. J. W. Male infant, aged 9 weeks.

Morbid Anatomy. Liver was small and contracted dark green in colour and very firm. The diaphragmatic surface was smooth, but the undersurface was nodular. Cut surface showed a uniform fine cirrhosis. Gall bladder and bile ducts were normal.

Histology. The normal architecture of the liver is upset to some extent by a diffuse interstitial type of fibrosis which spreads in between cords of hepatic cells and along the sinusoids in the form of fine wavy strands and bundles, isolating the parenchymal cells into small and large groups and columns. The parenchymal cells show varying stages of degeneration and necrosis. Many of the parenchymal cells are impregnated with a fine pigment of bile, and in the potential space between the hepatic cords plugs of inspissated bile can be seen. The necrosis is of a diffuse type involving single cells and small groups of cells. Kupffer cells are swollen proliferated and contain bile pigment.

There is a slight increase of fibrous tissue in the portal tract. The artery, vein and duct are

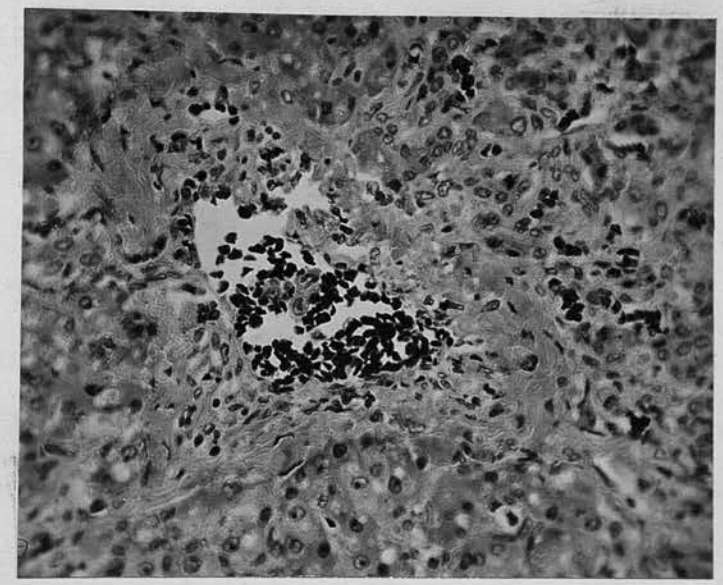

Fig XV. Liver of Case X. showing proliferative endophlebitis of a branch of the hepatic vein.

H and E x 275.

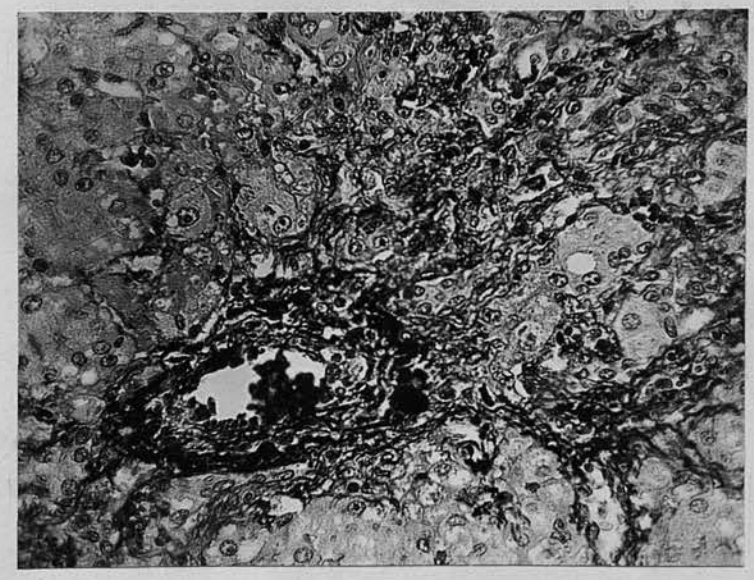

Fig. XVI. Liver of Case X. Highly cellular fibrous tissue is seen to permeate between hepatic cords & to extend~~ing~~ from a thickened central vein. The picture is characteristic of interstitial cirrhosis.

Azan x 300.

normal. There is no definite proliferation of biliary canaliculi but a few of them are elongated and tortuous probably a natural sequence to the slight increase of fibrous tissue in the sheath. The lumen of the bile ducts is patent and there is no disquamation of the epithelial cells nor is there any pericholangitis.

There is thickening and sclerosis of the hepatic venous tree.

Central veins show great fibrosis of their wall, from which extend outward many bundles of fibrous tissue. The larger branches of the hepatic veins show a definite inflammatory infiltration of their walls. The capsule is moderately thickened.

Case No. 11.

Name C. T. Female infant, aged 7 weeks.

Morphology. Liver was enlarged slightly, coloured dark green with a smooth surface. Cut easily. Gall bladder contained thick dark green bile. Bile ducts were patent.

Histology. The obvious change in the liver is an early interstitial increase of fibrous tissue which spreads from the portal tracts, and hepatic veins into the lobule. The cells of the parenchyma show degenerative changes. Their cytoplasm is deeply pigmented with bile, and their nuclei are irregular in shape and distribution and has affected single cells and isolated cords; but these patches of necrosis

seem to bear a closer relationship to the hepatic venous tree and its terminals than to the portal tract.

Portal tracts show a slight increase of fibrous tissue of a cellular character. The hepatic artery and the portal vein are normal. There is a definite, but not marked proliferation of bile ducts. The lumen of the ducts are patent, and there is no inflammatory cell infiltration.

Hepatic venous tree shows a moderate thickening of its wall, and this change has affected the hepatic terminals (central veins) as well. Narrow bands of fibrous tissue can be seen to proceed from the central vein into the surrounding tissue. In the larger branches of the hepatic vein there is a slight round cell infiltration of the wall.

The Kupffer cells are immensely swollen, polymorphic, and are stained greenish yellow with bile.

Case No. 12.

Name. M. C. Female infant, aged 10 months.

Macroscopic Appearance. Liver was smaller than average, surface nodular, and of a light yellowish brown colour. It was tough to cut and on the surface can be seen numerous firm nodules separated by fibrous tissue. Gall bladder and ducts were normal.

Microscopic Appearance. Narrow bands of fibrous tissue extending from portal tract to portal tract

has divided the parenchyma into islands of varying sizes. Extensive fatty change has affected most of the liver cells throughout the lobule. The cytoplasm and nuclei are well stained. There are no areas of necrosis. No hyaline change recorded by Mallory as being characteristic of portal cirrhosis can be seen in the neighbourhood of the Glissons Sheath,

Portal Tract. There is a slight increase of fibrous tissue in the portal tract, and this extends beyond the capsule to link up with the adjacent portal tracts. The resulting picture is that of a multilobular cirrhosis. The walls of the portal veins are relaxed and the lumen is distended with red cells. There is no sclerosis of the portal vein or hepatic artery. The bile ducts are patent and healthy. Proliferation of the bile ducts is slight. A few inflammatory cells can be observed in the portal tract and in the parenchyma abutting on it. Islands of regenerating liver cells are scarce, but a few such islands containing multi-nucleated liver cells, and cells showing mitosis are seen.

The sinusoids are collapsed and empty.

The Kupffer cells are impregnated with finely granular bile pigment.

Hepatic venous tree shows no thickening of the coats and there is no inflammatory cell infiltration.

Case No. 13.

Name. E. M. Female Infant, aged 3 months.

Morphology. Liver was greatly enlarged, light yellowish green in colour with a finely nodular surface. It had a firm consistency, was tough to cut having a sharp edge, and the cut surface suggested a monolobular cirrhosis. Gall bladder was healthy and contained bile. There was no obvious obstruction in the bile ducts.

Histology. Loose cellular fibrous tissue extending from the portal tract has encircled masses of liver cells, isolating them into lobules. There is some invasion of the lobules by fine strands of collagen fibres; but it is nowhere so extensive as to distort the parenchymal pattern. Most of the liver cells show marked fatty degeneration and a few are necrotic. Plugs of bile thrombi are seen in between the hepatic cords. A few of the liver cells and Kupffer cells are loaded with bile pigment.

Portal tract. There is moderate increase of fibrous tissue of a spongy and cellular type in the Glissons capsule. It extends from each portal tract to the adjacent ones in narrow bands in which are numerous proliferated biliary canaliculi. Lymphocytic infiltration in these fibrous bands is slight. The artery, vein and duct are normal. There is no thickening or sclerosis of the coats of vessels. The lumen of the bile ducts is patent; but many of the newly formed biliary canaliculi contain inspissated bile. The

hepatic veins, and the central veins do not show any abnormal changes. There is no phlebosclerosis nor endophlebitis.

Case No. 14.

Name J. N. Female infant, aged 14 weeks.

Macroscopic Appearance. Liver was much enlarged greenish yellow in colour, with a finely granular surface. Its consistency was firmer than normal, cut with resistance and the cut surface indicated a slight increase of fibrous tissue. A few nodules of yellow liver substance stood out clearly. Gall bladder was normal and contained bile. The ducts were patent.

Microscopic Appearance. The parenchyma is oedematous with great distension of the lymph space of Disse, and collapse of the sinusoids. Many of the cells appear rather foamy and the differentiation between individual cells is more marked than normal. Most of the nuelei are well stained, but here and there some of them show Karyorrhexis and lysis. An occasional bile thrombi is seen in some of the hepatic cords. A few widely distributed foci of haemopoesis are revealed on close observation.

Glissons Sheath. There is a slight increase of fibrous tissue of a loose and vascular character with scanty round cell infiltration. The arteries, veins and ducts appear normal.

The hepatic veins show no definite change of the wall and the central veins are normal.

of some lobules. This is probably an early case of cholangitis lenta described by Nauyn and St. Klein; where there is diffuse pericholangitis but where the periductal sclerosis has not yet brought on any biliary stenosis, or stagnation of bile.

Case No. 16.

Name. T. P. Male child, aged 8 months.

Morphology. Liver slightly enlarged. Bile stained to a deep green colour. Surface coarsely granular. Section showed dense fibrosis in a close network. Gall bladder was absent. Only a mass of fibrous tissue was seen lying at the posterior end of the gall bladder fossa. There was no common bile duct. The condition was congenital obliteration of the biliary passages.

Histology. The structure of the liver is not upset to any remarkable extent. The central veins are patent, and there is no necrosis at the centre of the lobules; but numerous liver cells scattered throughout the lobule show icteric necrosis. Their cytoplasm is granular, has taken the greenish yellow tinge of bile, and their nuclei have disappeared. This wide spread necrosis of isolated hepatic cells in the lobule has resulted in a certain amount of interstitial or intercellular fibrosis. There are numerous giant liver cells resembling foreign body giant cells with ten or more nuclei in them. No mitosis is seen. Wherever there is necrosis of cells polymorphs are much

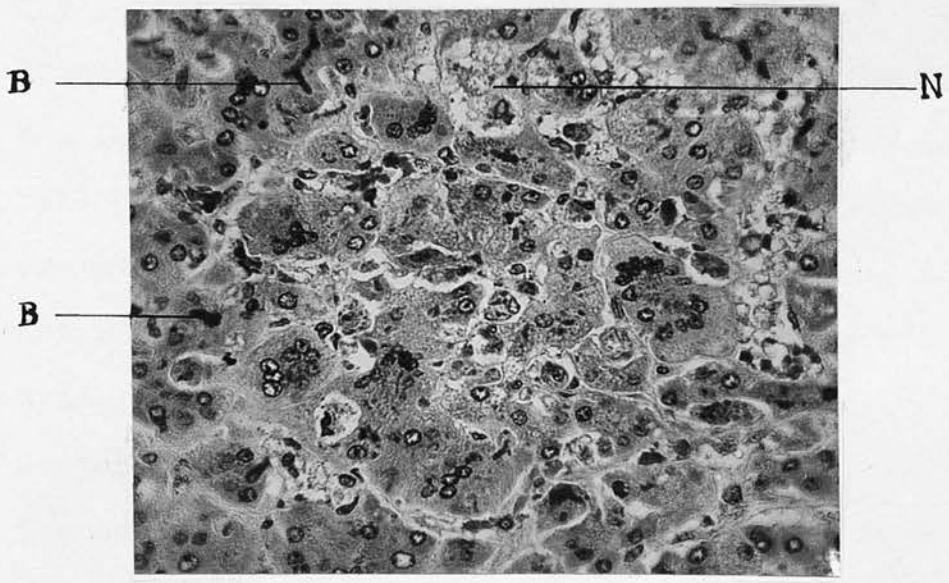

Fig. XVII. Liver of Case XVI. with congenital obliteration of bile duct. Numerous Multinucleated liver cells are seen.
B. Inspisated bile in the lumen of the hepatic cords. N. Necrosis of parenchymal cells

H and E x 300.

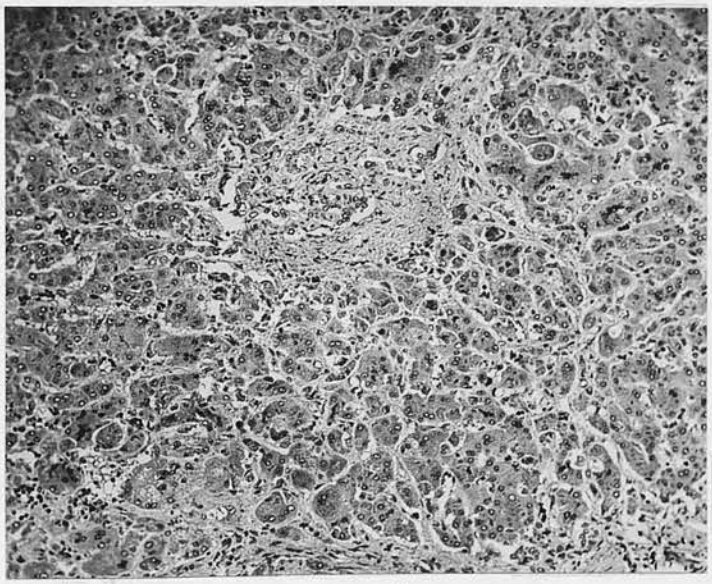

Fig. XVIII. Liver of Case XVI. Showing marked increase of fibrous tissue in the portal tract and slight interstitial fibrosis.

H and E x 110.

in evidence.

The portal tracts show a great increase of fibrous tissue, which has resulted in marked narrowing of the lumen of the portal vein. The absence of normal bile ducts in the Glissons Sheath in spite of the enormous increase of proliferated biliary canaliculi is noteworthy. Here and there bands of fibrous tissue bridge across adjacent portal tracts destroying the normal architecture of the parenchyma to some extent. The specific connective tissue stain demonstrates the compact bundles of fibrous tissue irregularly disposed in the portal tract, from which delicate strands of connective tissue permeate into the neighbouring lobules along the course of the sinusoids. There is periarteritis and narrowing of the lumen of the hepatic artery.

No alteration is seen in the hepatic venous tree. There is no thickening of its branches, nor is there any increase of fibrous tissue in the adjacent region.

There is increase of biliary canaliculi in the portal tract. A few are branched and tortuous showing aneurysmal dilatations plugged with inspissated bile. It is apparent that they are in direct communication with the hepatic cord which drain the bile into them; but not with the bile duct.

The Kupffer cells are swollen and are packed with bile pigment.

C O M M E N T.

After a study of the histopathology of these sixteen cases it is found that they can be classified under five different heads depending on the similarity of features in each group of cases. It was made certain from the report on clinical investigations of these cases as well as from the post mortem findings that syphilis can be definitely excluded as an etiological factor. Infection with micro-organisms is also ruled out as all the sections except two, cases IX. and XIII. for which paraffin blocks were not available were stained and examined for organisms with negative results.

Group I. Comprising the largest number of cases I. to XI. have the following features in common.

1. Inflammatory cell infiltration of the larger divisions of the hepatic veins.
2. Fibrous thickening of the hepatic veins and their terminals.
3. Intercellular, or interstitial and annular type of fibrosis.
4. Proliferation of biliary canaliculi.
5. Bile stasis.
6. Necrosis and regeneration of the parenchyma.

Group II. Cases XII. and XIII. fall under this head and the similarity of features are :

1. Extension of fibrous tissue from portal tracts to encircle large masses of liver cells.
2. Absence of noticeable change in the wall of the vessels.
3. Absence of inflammatory reaction in the bile duct.

4. No apparent necrosis of liver cells.

5. Marked fatty degeneration of cells.

Group III. Case No. XIV. has the following characteristic features :-

1. Oedematous distension of the lymph space of Disse.

2. Collapse of the sinusoids and attenuation of the hepatic cords.

3. Loosening of the cells in the cord, resulting in breaking up of the trabecula.

4. Early collagenous transformation of the reticulum of the sinusoids.

Group IV. Case No. XV. This case like the previous one is a precirrhotic condition. The chief changes in this liver were :

1. Periductal inflammatory cell infiltration.

2. Periductal fibrosis.

3. Periarteritis with considerable narrowing of the lumen.

4. Periphlebitis of the portal vein.

5. Marked fatty change in the parenchyma.

Group V. Case No. XVI. This was a definite case of congenital obliteration of bile ducts showing :

1. Increase of fibrous tissue in the Glissons Sheath and between the hepatic cords to some extent.

2. Obliteration of the lumen of bile ducts.

3. Absence of periductal inflammatory cell infiltration.

4. Icteric necrosis of liver cells.

5. Periarteritis and Periphlebitis of the portal vein.

Of these five groups the first one possesses features which are common to the six cases of infantile cirrhosis of India as well as to five cases from Edin-

burgh, and this group will be discussed in detail later.

Group II. The histopathological picture of cases XII. and XIII. are typical of early Laennec's cirrhosis before vascular changes have set it. All aspects of the histology of this type of cirrhosis has been so well studied and clarified, that it does not require more than a passing reference.

Group III. Case XIV. is an example of serous hepatitis described by Rossle and Eppinger.

Group IV. Case XV. This is the second case in this series of sixteen cases with ulceration of the cornea. The association between degenerative changes in the liver cells and corneal ulceration is significant in view of the important rôle assigned to the liver in vitamin A storage. The histology is characteristic of the precirrhotic stage of chronic cholangitis described by St. Klein and Naunyn and reported by Rossle. Preservation of architectural pattern, increase of fibrous tissue in the portal tract and periductal inflammatory cell infiltration are the outstanding points.

Group V. Case XVI. Showed naked eye and microscopical evidence of obliteration of bile ducts of non-inflammatory origin.

Discussion on Group I.

Pathogenesis of phlebitis and phlebosclerosis of the hepatic venous tract.

The literature on inflammation and sclerosis of the venous system of the liver is scanty, and it is especially so of hepatic veins and their radicles. Borrmann (1897) was one of the first to observe endophlebitis with thrombosis of the portal vein. Hess A. F. (1905) has given an account of a study of 23 cases fatal obliterating endophlebitis of the hepatic vein. He suggests that besides syphilis inflammatory toxins of other nature can produce a similar effect. Wohlwill (1925) observed portal sclerosis and thrombophlebitis in 16 of the cases he studied. Simmonds (1912) studied 7 cases with phlebosclerosis of the portal vein and hyaline changes in the intima. He differentiates a primary or inflammatory type caused by syphilis and a secondary type the result of increase of pressure in the portal vein as occurs in portal cirrhosis. Kretz Richard in his study of portal cirrhosis describes the sclerosis of the portal vein, and the scarcity of portal and hepatic venous terminals. McIndoe (1928) in his investigations on the vascular system of the liver in portal cirrhosis by corrosive technique found sclerosis and distortion of the portal and hepatic veins. McMichael (1934) found sclerosis of the intima and hypertrophy of the media in the portal and hepatic systems in cases of cirrhosis and concluded that it was mainly due to

increased venous presence in the portal system. Coronini and Oberson describe cases with obliterative endophlebitis of the hepatic vein resulting in collapse of the sinusoids and the formation of pseudolobulations. Cronini (1939) in his investigations on cases of endophlebitis of the hepatic vein in cases of cirrhosis in children classes this condition with a rheumatic or pseudorheumatic state, as he found Aschoff bodies in the liver heart and other organs. Radhakrishna Rao (1935) from a study of five cases of infantile biliary cirrhosis suggests a vaso-motor disturbance due to toxins in the circulation which results in vaso-spasm of the hepatic veins, and a dilatation of the portal vein, as the primary cause which leads to phlebosclerosis of the hepatic vein. Wurm (1939) who reports a histological s tudy of eight cases of endophlebitis of the hepatic veins and their terminals in infants three to four months old, finds a sub-endothelial collection of serous fluid and later a fibrillary increase in the wall of the central and sublobular veins. He found no inflammatory changes in the vessel wall or in other viscera, and there was no cirrhosis of the liver.

In the present study in nine cases out of the eleven examined under this group, a definite inflammatory reaction of the radicles of the hepatic veins was a constant finding. In the other two (cases III. and IV.) there was no obvious cell infiltration of the hepatic veins in spite of the marked thickening of the

wall. The inflammatory cells were chiefly lymphocyte, plasma cells and histiocytes laden with granular bile pigment. In cases I. and X. this cell infiltration was more marked in the intima whereas in the others it affected all the coats. Phlebosclerosis and endophlebitis were present in all the eleven cases, but varied in degree. It was most marked in case III. where some of the smaller branches of the hepatic veins have undergone complete obliteration of their lumen, and the larger ones show an enormous thickening of the intima reducing the calibre of the lumen considerably. It was observed that when the inflammatory reaction was marked, the sclerosis was less evident, and vice versa. Fibrous tissue was more abundant in those cases with marked endophlebitis and obliteration of the lumen.

Day in his study of eleven cases of cirrhosis of the liver due to raised venous pressure found thickening of the branches of the hepatic veins and fibrosis round the larger branches of the hepatic veins as well as in the portal tract. In the series of cases under discussion there was some similarity in the distribution of the fibrous tissue to that reported by Day (1940); but there is no similarity in the changes in the hepatic veins since they showed in nine of these cases a marked inflammatory infiltration of the wall. There was no lesion in the heart or elsewhere to account for a raised venous pressure. Radhakrishna Rao (1935-36) maintains that the stenosis

of the hepatic venous tree is the result of a primary vascular disturbance in the branches of the hepatic vein due to a vaso motor imbalance in the liver. A similar explanation is given by Sulzberger (1933) for thromboangietis obliterans which is usually associated with specific and marked hypersensitivity of the vascular apparatus of the skin to tobacco. This is a possibility in cases of cirrhosis with central necrosis only; but the widespread sclerosis and inflammatory reaction in the hepatic veins cannot be overlooked in these cases. Coronini attributes this change to a toxic or infectious process. Infection can be ruled out, as these sections did not show the presence of bacteria. In view of the fact that endophlebitis and phlebosclerosis of the hepatic vein may occur without a co-existing cirrhosis of the liver (Wurm) we may decide that the lesion in the hepatic vein is not decidedly the cause of cirrhosis; but that it is incidental, and may contribute to the liver changes in the later stages. It is difficult to visualize the hepatic veins bearing solely the stress of a haematogenous toxin brought to the liver through the portal vein or the hepatic artery without producing sclerotic changes in these vessels. Cameron and Karunaratne (1936) suggest that in cases of cirrhosis of the liver the product of autolysis of the hepatic cells is drained by the lymph spaces to the portal tract where they may stimulate fibrolastic growth. The pathogenesis of the sclerosis and the inflammatory infiltra-

tion of the hepatic veins seem to have an analogy to this explanation of cirrhosis, It is probable that in these cases autolysis of the hepatic cells is more extensive, and the location of the parenchymal necrosis (central) bears a closer relationship to the hepatic venous tree so that the products of autolysis act through the blood, directly on the hepatic veins. The finding of bile-pigment laden histiocytes in the wall of the hepatic vein lends support to this view.

Genesis of fibrosis.

It was pointed out by Mallory (1911) that simple necrosis of liver cells does not result in proliferation of connective tissue when the blood vessels and the connective tissue stroma are uninjured, but the connective tissue which is there collapses and thickens resulting in an apparent but not real increase of fibrous tissue. Whereas when infection is present the liver cells and fibroblasts are destroyed and there is excessive formation of fibrous tissue. This idea has been elaborated by other observers notably by Moon in his work on experimental cirrhosis. A divergent view was expressed by Lacquet (1932) in his experimental work on liver. He found that connective tissue production in experimental cirrhosis with carbon-tetra-chloride intoxication was not dependent on necrosis of liver cells. In recent years evidence has been accumulating to place the responsibility for the formation of collagenous fibrous tissue on the less deferentiated mesenchymal cells.

Doljanski and Roulet (1933) from their tissue culture work suggest that the mesenchymal cells secrete a diffusible substance which stimulates the formation of collagenous fibrils from the tissue fluids. Joan Ross from her study on the pathology of reticular tissue concludes that reticular syncytium are under certain circumstances changed into fibrocytes, and that under pathological conditions the argyrophil fibres may be converted into collagen.

Whatever be the type of cell responsible for the transformation of reticulum into collagen fibres it is apparent that the permanent collapse of the sinusoidal bed and the abundant reticulum that is normally present in the liver are responsible for this type of fibrosis.

In the present investigation it was noted that the fibrous tissue was loose, vascular often containing compressed hepatic cords, and biliary canaliculi in its meshes. It is evident that the mechanism of the production of fibrosis is first the necrosis of parenchyma, its autolysis and removal through the lymphatics as well as through the hepatic tree leading to a collapse of the sinusoidal bed. This collapse of the "skeleton of the old lobule" was noticed by McDonald and Milne (1909) in their study on sub-acute liver atrophy. Later a gradual conversion of the collapsed sinusoids into collagenised connective tissue takes place. This change is brought about in

the presence of lymph rich in protein content by the uninjured fibroblast or Kupffer cells which according to Nathen (1908) are the source of fibroblasts in cirrhosis of the liver. The resulting band of loose fibrous tissue is augmented and widened when the adjacent regenerating parenchyma necrose in their turn with subsequent collapse of their sinusoids. The annular fibrosis seen around many of the pseudolobulations is the result of compression of the collapsedand sclerosed reticulum fibres by the regenerating parenchyma. It is now an accepted fact that there is a pericapillary lymph space (Krough 1922). Starling's investigation on the formation of lymph in mammals showed that in the liver and intestine the capillaries are so permeable normally to protein that effective osmatic pressure becomes less than the capillary blood pressure. In fact the protein content of the lymph in the liver reaches very nearly that of inflammatory transudate. The sclerosis of the hepatic veins and their terminals tends to increase the intra-capillary blood pressure in the liver so that the production of lymph is greatly increased. In case No. III. it can be observed that the hepatic venous radicles show enormous thickening of the wall leading almost to obliteration of the lumen and in the fibrous tissue can be seen numerous dilated lymphatics. It is this increase in the quantity of lymph with a high protein content and mixed with products of autolysis of liver cells that forms the subtrate which stimulates the production of interstitial collagenous fibrils.

The bile pseudo canaliculi.

A marked proliferation of the canaliculi were observed in nine of the eleven cases studied. In case IX. the proliferation was slight and in case X. it was absent. In these two cases the type of fibrosis was more interstitial than annular or interlobular. The formation of bile pseudo canaliculi in cirrhosis and their genesis has always been a matter for controversy. These structures were first observed by Wagner in 1862. MacCallum and Schoppher held that these ducts grew from pre-existing interlobular bile ducts and took part in the regeneration of liver cells along with the spared liver cells. This idea was supported by Meder, Stroebe and others who reported observing the transition stages. Rolliston and McNee considered them as hepatic cords detached from the lobular periphery and compressed by fibrous bands. Craven Moore (1908) regarded them as a transitional type of liver cells. McDonald and Milne (1909) record that in their five cases of sub-acute liver atrophy there was no evidence of the bile ducts developing into liver cells ; a finding which tallies with that of Cameron and Karunaratne (1936). Miller and Rutherford (1923) Boyd (1939) and Mallory hold a similar view. Findlay (1900) is of opinion that these are the result of a reversion to the embryonic type of liver cell during the process of regeneration.

In the cases under consideration the bile pseudo-canaliculi were observed in great profusion in those

cases where destruction of liver cells occurred en masse and were less in evidence where the destruction was localised to smaller groups of cells. In cases V. VI. IX. and X. the cirrhosis was more intercellular in type than in the others, and the necrosis affected smaller groups of cells and isolated hepatic cords. The proliferation of bile pseudocanaliculi was less noticeable in case V. and in case X. it was not recognisable at all. When we consider the rich arterial supply of the bile ducts, and the canals of Hering (Cameron and Meyes) it is quite reconcilable with the finding that the bile ducts in the necrotic areas are not involved in the general destruction of the parenchyma, but persist and become apparent in the collapsed reticular framework when the necrotic debris is removed. The epithelium of the ducts are said to be more resistant to the action of injurious agents than liver cells. It must also be noted that actual sprouting of bile canaliculi from pre-existing duct epithelium does take place to some extent in cirrhosis. It was observed that in areas of massive necrosis, for no apparent cause a few attenuated hepatic cords survived, which later were still further compressed by the collapsed and sclerosed reticulum fibres so that ultimately they resembled very closely the bile-pseudo canaliculi.

Bile Stasis. The causation of bile stasis in these cases in the absence of either catarrh of the bile ducts or pericholangitic stenosis needs explaining. Case II. showed periductal fibrosis and some inflammatory infiltration of the periductal region, without any obvious occlusion of the lumen, or catarrh of the lining epithelium. In the other ten there was no sign of inflammation of the ducts or obliteration of the lumen, yet there was wide spread pigmentation of the liver cells and Kupffer cells with bile; and plugs of inspissated bile were observed in all cases in the potential axial lumen of the hepatic cords. Althausen T. L. (1931) in his study of a number of cases of toxic cirrhosis demonstrated clinically that in toxic cirrhosis there was normal carbohydrate metabolism as shown by dextrose tolerance test, and at the same time marked retention of the dye in Rosebengal dye excretion test. The latter test is based on the fact that there is selective elimination of the dye via the bile channels when it is given intravenously. He assumed that as a result of the loss of original architecture of the liver in this disease the regenerated parenchyma lacked connection with bile channels. This accounted for the bile stasis and for the impaired excretion of the dye. Milne (1909) also records having observed in his cases that many of the biliary canaliculi communicated either with the interlobular bile duct or with regenerated hepatic cords. In many of the cases

under discussion bile thrombi were observed in the bile pseudo canaliculi which evidently communicated with the hepatic cords; but not with the duct. Jaundice was a constant clinical finding in all these cases. It was this feature in the histology of the cirrhosis of infants in India that earned it the misnomer of "Biliary cirrhosis" from pathologists notably Gibbons who examined these cases first. The pseudolobules were formed from hyperplastic, bile stained regenerated parenchyma. The normal trabecular arrangement of the lobule was lost, and there was absence of central vein. Since the liver cells do not regenerate from bile duct epithelium it is not surprising that the outlet for bile from the regenerated cords is imperfect. Whether in course of time the bile canaliculi will succeed in establishing drainage from the newly formed hepatic cords to the bile ducts is a question which requires investigation.

Necrosis of the parenchyma.

It was Mallory (1911) who first described toxic cirrhosis where necrosis of liver cells occurred around the hepatic vein, (central necrosis) as a result of the action of toxins in the circulation. Though he does not record any changes in the blood vessels yet the distribution of necrotic foci, and the type of fibrosis that follows in the cases under review bear much resemblance to that described by Mallory. These eleven cases present different stages in the evolution of cirrhosis and vary in the extent

of damage to the parenchyma. It will be seen that in the less chronic cases (cases VIII. and XI.) the damage is central in distribution while the cells around the portal tract are not affected at all. Where the cirrhosis is far advanced it is possible to infer with accuracy from the distribution of fibrous tissue around the hepatic venous tree, and the scarcity of the radicles of the hepatic vein that the degeneration had first affected the centre of the lobule leading to its collapse and obliteration of the central vein. As stated previously the sclerosis and endophlebitis of the hepatic veins are not primarily the cause of necrosis of the liver cells; but they interfere in the later stages with the free outflow of blood from the liver. Under physiological conditions the liver which is well innervated by sympathetic and parasympathetic systems is able to control its vascular supply to a remarkable extent. It is inferred that sclerosis of the hepatic vein up to a certain degree does not interfere with the normal functions of the hepatic parenchyma; but beyond which the liver cells are not able to cope even with its normal functions, because of the permanent vascular upset. This apparently is the irreversible cirrhotic stage of Cameron and Karunartne, and the decompensated cirrhosis of Chapman, Snell and Rountree, where the liver cells fail to regenerate and where massive necrosis of liver cells involving whole lobules occur. It is in these cases with marked fibrosis

that lobules of regenerated liver cells are observed to have undergone necrosis en masse. The additional factor which contributes to this degeneration of parenchyma is the imperfect drainage of bile from the hepatic cords. Frank C. Man observed that dogs whose bile duct had been ligatured previously failed to show the same remarkable regeneration of liver tissue after lobectomy as that occurred in normal liver. He found that interference with circulation in the liver or the outflow of bile hindered regeneration to a greater extent than the mere presence of excess of fibrous tissue. In this type of toxic cirrhosis in infants it is the interference with the exit of blood from the liver by the sclerosed and stenosed hepatic veins and the imperfect drainage of the regenerated cells that add to the parenchymal damage in the later stages.

The nature of the toxin and the mode of action.

This has been studied in detail in the experimental work on liver damage in the progeny; but it will not be out of place here to recapitulate some of the discussions and findings. It was mentioned before that in this group of cases the possibility of an infection in the liver or a syphilitic cirrhosis was excluded. Liver has a remarkable resistance to infection and the role played by the well developed system of reticulo-endothelial cells in combating infection cannot be over-estimated but the rich blood

supply which acts as a deterrent to infection is probably the very factor that makes the liver susceptible to toxins of various types. From the work of Starling (1894) it is known that the capillaries of the liver and intestine are normally permeable to some proteins. Subsequent work by Sabin and Band (1926) and by Stillwell (1926) show that the capillaries of the liver are normally much more permeable to dissolved substances of a colloid nature than those of other organs. This permeability of the endothelium of the sinusoids to molecules of greater dimension and the comparatively sluggish circulation in the liver are factors that place the liver more vulnerable to toxins in circulation than any other organ in the body. Liver possesses a detoxicating action on injurious agents and it is also the clearing house for products of absorption in the intestine. Since the capillaries of the intestine are equally permeable to substances of a colloid nature it is probable that the toxin is an agent absorbed in alimentary canal. The danger of naturally occurring toxins being absorbed through the intestine and damaging the liver must not be overlooked. Toxic cirrhosis of the liver due to ingestion of cinchophen, (Weir and Comfort, and Henry H. Kramer)hydrocin (Ingham) Senecionine (Davidson J.) and Copper (Mallory F.B.) have been reported. It is interesting to note that wheat grown in certain soil containing selenium absorbed enough of the element to produce pathological changes in the liver of the live stock fed on it (Kurt W. Franke 1934).

Recent work by Gyorgye and Goldblatt and by Arnold R. Rich and John D. Hamilton show that a well balanced diet lacking only in a special type of protein will produce necrosis of the parenchymal cells and cirrhosis in the livers of rats and rabbits. The active principle or principles that were lacking in this diet and which when present protected the liver against chloroform and carbon-tetra-chloride were identified by the work of Forbes, Neale and Shearer (1936) Forbes and McConnell (1937), Forbes (1938) Neale and Winter (1938) and Fritzbargh (1939). They were found to be of the nature of purine bases, the most important of which was prepared from liver and later identified as xanthine. From the present study of "infantile biliary cirrhosis" of India in relation to its distribution and dietetic habits of the mothers it was found that the diet of the people in those areas where the disease occurred consisted chiefly of polished rice and some vegetables and lacked in nucleic acid and proteins. Most of them abstained from taking meat and the six cases of infantile cirrhosis from India included in the present study are from families of vegetarians.

It is safe to conclude that two factors are involved in the production of necrosis of the parenchymal cells of the liver and the evolution of cirrhosis. One is the protective factor in the diet in the absence of which a second factor, the toxin, probably absorbed from the intestine causes degenerative changes and necrosis of the liver cells. The protective factor in

the diet is a purine base. The nature of the second factor the toxin is not yet known. From the experiments of Gyorgye and Goldblatt, and of Rich and Hamilton it is assumed that it is a toxin normally absorbed from the intestinal tract and detoxicated in the liver in the presence of the protective factor. In the absence of this protective factor necrosis and later cirrhosis results.

Five of the eleven cases in this series showed a definite familial tendency. Experiments on placental permeability prove that a cirrhogenic toxin can pass through the placental barrier and injure the liver of the foetus. It also passes through the mammary secretion and causes necrosis of the liver of the sucklings in the absence of the protective factor in the diet of the mother. A certain minimum amount of this protective factor must be present in the mother's diet to safeguard the liver of the suckling against a known cirrhogenic toxin administered to the mother.

It is possible that in these cases of infantile cirrhosis of the liver, especially in those with a familial tendency the etiological factors are either an absence of the protective factor in the form of nucleic acid and purine bases in the diet of the mother, or a faulty digestion and failure of absorption of these substances in the mother or infant or an abnormal metabolism with an inability to utilize absorbed material.

C O N C L U S I O N.

Cases of cirrhosis of the liver resembling in histopathology "infantile biliary cirrhosis of India" occurs occasionally among infants of Edinburgh. The cirrhosis is classified as a toxic cirrhosis.

S U M M A R Y.

A study of the histopathology of 6 cases of infantile biliary cirrhosis of India, 9 cases of cirrhosis of unknown etiology from the Royal Hospital for Sick Children Edinburgh and one case of cirrhosis due to congenital obliteration of the bile duct from the same hospital has resulted in the following findings.

(1) Five of the cases from Edinburgh show histological features similar to that found in the six cases of infantile cirrhosis from India.

(2) Endophlebitis with sclerosis and thickening of the hepatic venous tree was the outstanding characteristic of all the eleven cases, and inflammatory cell infiltration of the radicles of the hepatic veins was marked in nine of these.

(3) The cirrhosis is classified as a toxic corrhosis from the histological study.

The etiology of toxic cirrhosis is discussed.

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